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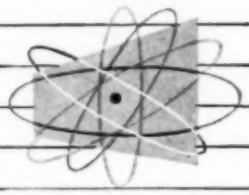
Pharmaquiz©

1. How many non-government buildings stand on Constitution Avenue in Washington, D.C.?
   a. None
   b. One
   c. Three



2. Uranium was first identified by:
   a. A Nobel-prize chemist
   b. An Austrian geologist in 1847
   c. An 18th century pharmacist

3. "Compoundable" chemicals are:
   a. Of uniform bulk
   b. Of unquestionable purity
   c. Custom built for pharmacists

ANSWERS

1. b. One—The building of the American Pharmaceutical Association.
2. c. Uranium was first identified by M. H. Klaproth in the laboratory of his retail pharmacy in 1789.
3. a. b. c. "Compoundable" chemicals live up to all three of these specifications and bear the Mallinckrodt label. The uniform, dependable purity of Mallinckrodt products is a by-word among pharmacists. Mallinckrodt prescription chemicals are eminently "Compoundable."

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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A Three-Hour "Physical Development" Cup-Plate Assay for Penicillin*

By FRANK M. GOYAN, JEAN DUFRENOY, LOUIS A. STRAIT, and ROBERTSON PRATT, with the Laboratory Assistance of TOINIE JUNTUNEN†

A practical assay method for penicillin that can be completed in three hours is reported. The procedure retains the advantages inherent in the cup-plate method, but reduces the delay necessitated by the sixteen- to eighteen-hour period of incubation customarily employed. Other advantages of the new method are that it yields a linear calibration curve and that the curve has a useful range nearly twice that of the conventional method. The procedure employs a principle of physical development that is believed to be new in biological assays. The seeded layer of test plates is rendered analogous to a photographic emulsion and is treated as such.

THE PROCEDURE reported here is one outcome of a study of certain aspects of the interrelationship between the biological and physical phenomena involved in the assay of penicillin by the cup-plate method. During the course of investigating systematically the effect of several physical factors on the size of inhibition zones produced by different concentrations of penicillin against *Staphylococcus aureus*, NRRL Strain No. 313 (same as F.D.A. Strain No. 209P) means were sought to intensify the extremely faint zones that are visible on the plates after five hours of incubation. Among the methods employed were staining with various dyes and impregnation with metallic substances. Among the metals, silver seemed promising because of the observation (1, 2) that normal cells of *Staph. aureus* and those under the influence of penicillin differ in their affinity for silver supplied in aqueous solutions of silver nitrate.

The assumption was made that, after impregnation with silver, the seeded layer of incubated plates would be analogous to a photographic emulsion in that light would act upon it to produce a latent image. Accordingly, seeded plates prepared as prescribed in the specifications of the Food and Drug Administration [similar to the recommendations of Schmidt and Moyer (3)] were treated with silver nitrate, then were exposed to light, and finally were subjected to

* Received Nov. 27, 1946, from the University of California College of Pharmacy, The Medical Center, San Francisco, Calif.

† Acknowledgment is made to the Cutter Laboratories, Berkeley, Calif., for a generous research grant that made the execution of this work possible.

a technique of physical development essentially equivalent to development of a latent image on a photographic emulsion. This technique produced images which showed a marked difference between zones of inhibition and noninhibition with a clearly defined boundary between the zones after only five hours of incubation. In subsequent experiments, sharply defined images were obtained after two and one-half hours of incubation following the addition of penicillin provided the seeded plates were previously incubated for three hours before penicillin was added. The diffusion of penicillin was found to be sufficiently extensive in the shortened second period of incubation to produce 15 mm. to 16 mm. zone diameters at a concentration of penicillin of 1 unit/ml. as compared to 20 mm. to 21 mm. zones produced by the standard method requiring an incubation period of sixteen to eighteen hours. The pre-incubated plates may be stored in a refrigerator for several days without affecting the result of the assay. The entire procedure results in a three-hour cylinder-plate assay which retains the numerous advantages inherent in this technique but eliminates the serious disadvantage of the standard method, i.e., the relatively long period of incubation. A photograph of a typical plate processed by the rapid method is reproduced in Fig. 1. Some definition has been lost in photographing and reproducing it.

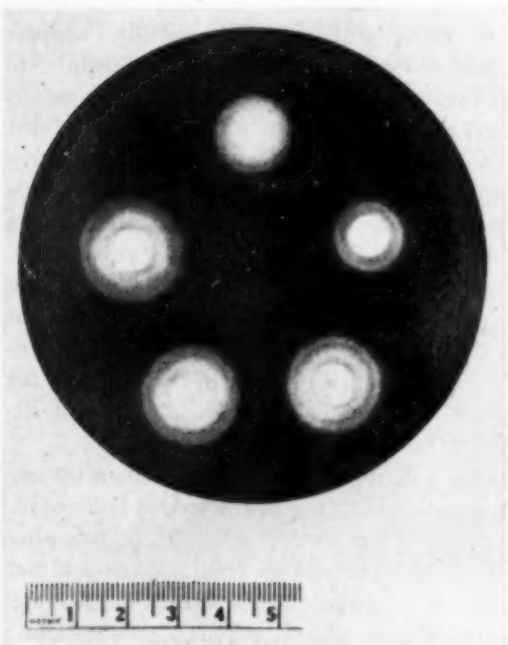

Fig. 1.—Reproduction of a photograph of a typical penicillin assay plate processed by impregnation with silver and physical development after three hours of secondary incubation and diffusion of penicillin. Zones for solutions containing 1, 0.5, 2, 4, and 8 units/ml., respectively, are arranged in clockwise order beginning with the one at the top.

The zones of inhibition on plates processed by physical development after short incubation periods are not of uniform density but reveal definite concentric circular light and dark areas. They are a constant feature of this method but the explanation for them is not clear at present. It is the diameter enclosed by the boundary between the outermost light area and the surrounding dark background that is measured. This boundary is generally orange or yellow in color on freshly processed plates but tends to fade after several hours.

Numerous other short interval assays for penicillin have been proposed (4–9) but we believe that the present method warrants serious attention because the cylinder-plate technique is used most widely for routine assays of penicillin.

EXPERIMENTAL

The three-hour method used in our laboratory is as follows:

Preparation and Incubation of Plates.—1. Plates are prepared and seeded as for the standard cup-plate method of assay.

2. The plates are incubated for three hours at 38°. This is the preincubation period without penicillin.

3. The plates are then placed in a refrigerator and stored a few hours or days until needed. Preincubated plates have been used with entirely satisfactory results after five days in a refrigerator.

4. When assays are to be made, standard penicylinders are added in which the solutions to be tested are placed.

5. Plates are then re-incubated (post-incubation) for three hours at 38°. (Satisfactory images may be developed on plates with two and one-half hours of post-incubation under favorable conditions, but for routine assays, plates with three hours of post-incubation are preferred because the boundaries between zones of inhibition and noninhibition are sharper.)

Physical Development.—After removal from the incubator, the plates are treated as follows:

1. Flooded with about 30 ml. of a nearly neutral 0.1% aqueous solution of silver nitrate.

2. Exposed for two and one-half to three minutes to the illumination from two 40-watt daylight fluorescent lamps mounted in a reflector[1] at a distance of about 35 cm. This is equivalent to approximately 350 foot-candles. Additional short exposures to ordinary laboratory illuminations are not detrimental.

3. Excess silver nitrate solution is removed using a suction device.

4. Thirty milliliters of physical developer are then added and allowed to remain for from seven to ten minutes at room temperature.

5. The diameters of the zones of inhibition may be read in the conventional way.

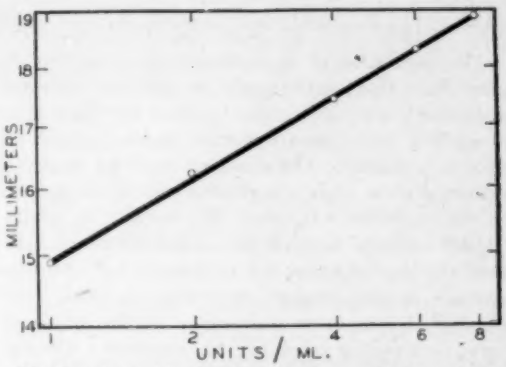

Fig. 2.—A standard calibration curve for the three-hour cylinder-plate assay for penicillin. (See text for explanation and description of curve.)

TABLE I

Preparation[b]	Series A: Potency[a] Determined by 3 Hour Cup-Plate Assay (2½ Hours of Post-Incubation) Value Read from		Series B: Potency[a] Determined by 3½-Hour Cup-Plate Assay (3 Hours of Post-Incubation) Value Read from		
	Standard 18-Hour Cup-Plate Assay u./mg.	Master Standard[c] u./mg.	Daily Standard[d] u./mg.	Standard 18-Hour Cup-Plate Assay u./mg.	Master Standard[c] u./mg.	Daily Standard[d] u./mg.
Commercial Na penicillin (No. 417 P-1)	1124 916	1152 1045	1109 1092	1052	1067	1071
Na penicillin G (No. HB551)	..	..	..	1417 1355	1538 1363	1243[e] 1325
Na penicillin X (Research Product)	..	..	..	775 775 776	788	761 765 775
Crude liquor (Run No. A-147)	..	..	..	753[f] 767[f]	816[f] (Figures from one plate only) 763[f]	783[f] (Figures from one plate only)
Na penicillin A-15 (Partly purified)	253	260 246	246	270 264 243	252 254 239	243 273 237
Ca penicillin (Partly purified) (No. 2 Std.)	346	349 337	..	..	..	..
Tablets[g]	..	..	..	729[g]	753[g]	648[g]

[a] Each figure represents average from four plates, except as noted. Series A and B run about four weeks apart. Duplicate assays (where given) within each series were done on different days.

[b] Samples of penicillin supplied by Research Division of the Cutter Laboratories. Samples Nos. 1, 2, and 4 from fermentation by *Penicillium chrysogenum* Q176.A8; No. 3 from strain N22; Nos. 5 and 7 from *P. chrysogenum* X1612; No. 6 from *P. notatum 1240.B4*.

[c] Calibration curve based on Fig. 2. One standard (1 unit/ml.) used on each plate to locate curve.

[d] Calibration curve located by drawing line between points for two standards (1 and 4 units/ml.) on each plate.

[e] Average of three dilutions which gave poor agreement. Reason for lack of agreement unknown.

[f] u./ml.

[g] Compounded of acacia, stearic acid, lactose, and Ca penicillin Cutter at U. C. C. P. The preparations tested were suspensions of ground tablets in water—not solutions. This accounts for irregular assays.

Directions for preparation and use of developer:

Stock Solution A.—1. Dissolve 80 Gm. sodium thiosulfate and 20 Gm. anhydrous sodium sulfite in 300 ml. distilled water.

2. Dissolve 8 Gm. silver nitrate in 200 ml. distilled water.

3. Slowly pour solution 2 into solution 1 while stirring. The order of mixing and the stirring are important.

Stock Solution B.—Dissolve 20 Gm. sodium sulfite in 500 ml. distilled water and when solution is completed add 3.8 Gm. 2,4-diaminophenol hydrochloride (Eastman Kodak Co. No. P614) and stir until dissolved.

Just before use, mix equal parts of solutions A and B and dilute their combined volume with 3 volumes of distilled water. We have found this dilution best for our use, but the optimum concentration to be used for varying conditions of light intensity, temperature of development, etc., should be determined empirically in each laboratory. The stock solutions A and B should be stored in a refrigerator.

[1] General Electric Company, Catalogue No. 59G283.

RESULTS

The evaluation of an unknown concentration of penicillin is customarily made by interpolation from a standard calibration curve in which the diameters of zones of inhibition are plotted against concentrations of penicillin. The standard curve for the conventional plate assay is nonlinear even when plotted on log-log scales. It covers the range 0.25 to 4.0 Oxford units/ml. and has been generally useful only over the limited range 0.5 to 3 units/ml. An advantage of the present three-hour method with physical development is linearity of the standard curve on a log-log graph over the range of 1.0 to 8.0 units/ml. with but slight deviation between concentrations of 0.5 and 1.0 unit/ml. A standard curve typical of this method is given in Fig. 2. Each point on the graph is the mean of readings from 118 plates upon each of which was placed the series of concentrations plotted in the figure. The inherent validity of the linear relationship is indicated by the fact that these 118 plates were processed on different days and under differing conditions. Among the variables that were investigated briefly were length of exposure to light, time and temperature of development, *p*H of solutions, and density and depth of the seeded layer.

A comparison is given in Table I of potencies determined by the standard eighteen-hour cup-plate method and by the three-hour method. The penicillins used were of different degrees of purity and ranged from crude liquor to crystalline products.

In general the procedures used in the physical development method are not critical with the exception of the concentration of silver nitrate and the time of exposure to light. The concentration of silver nitrate must not be less than 0.1% nor more than 0.13% and the length of exposure must be adjusted empirically in much the same manner as is customary in adjusting the length of exposure of photographic materials. The quality of light used for exposure is not critical but excess of heat radiation must be avoided. Reasonable caution must be observed not to injure the seeded layer in adding or removing liquids such as the silver nitrate and the developer solutions. Penicillins G and X and mixtures of G and K were used and were all effective in producing an image on seeded plates treated as above.

In most of the assays recorded above, the cylinders were removed before adding silver nitrate solution. However, if the cylinders are removed and the residual solution contained in them is allowed to flow over the seeded surface, gentle agitation is required to avoid serious unevenness of development. Agitation is not required if the cylinders are left in place during the processing.

Attempts to evaluate the independent contributions of the component biological and physical factors which interact to produce zones of inhibition in cup-plate assays of antibiotics have been unsuccessful. The physical development procedure can be used to investigate the interrelationships of these factors. A study of the difference in the zones that are formed with a range of variable times of pre- and post-incubation may be used to untangle the physical diffusion from the growth factors which interact to produce the zones.

SUMMARY

This paper describes a practical procedure for assaying penicillin in three hours. This procedure retains the advantages of the cup-plate method but eliminates its serious disadvantage, i.e., the sixteen- to eighteen-hour period of incubation. Additional advantages of the present three-hour method are linearity of the standard calibration curve and extension of the useful range to nearly twice that of the conventional method. The calibration curve for the three-hour method covers the range from 1 to 8 units per ml. The present method applies the principles of photographic development to the seeded layers of the test plates which are rendered analogous to photographic emulsions by impregnation with silver.

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Influence of Phosphate on Stability of Partially Purified Penicillins*

By ROBERTSON PRATT†,‡

Low concentrations of a phosphate have been found to exert a stabilizing action, independent of buffering effect, on sterile water solutions of penicillin. Phosphates in the concentrations used did not influence the stability of dry penicillin but upon subsequent solution did protect it to some extent from thermal decomposition.

THE PROBLEM of improving the stability of penicillin in aqueous solutions is an important one for the pharmacist, the physician, and the producer, not to mention the ultimate consumer—the patient. This problem has been recognized almost since the time of Fleming's initial observation on penicillin in 1929 (1). Until comparatively recently, however, relatively little work on this subject has been published.

In 1945 a group of workers at the Northern Regional Research Laboratory reported the results of a comprehensive series of experiments in which the stability of a crystalline sodium salt of penicillin in aqueous solutions at different temperatures and at different H^+ ion concentrations was studied (2), and they observed that the destruction of penicillin in aqueous solutions is a first-order irreversible reaction. They observed also that under similar conditions of storage (*p*H 2.0 at 0°) the stability of a solution prepared with crystalline sodium salt was greater than that of a solution prepared with partially purified sodium salt. The earlier pertinent literature is reviewed in their paper.

Fermentation studies with *Penicillium notatum* in our laboratory suggested that a positive correlation, independent of buffer action, exists between the concentration of KH_2PO_4 initially present in the culture liquor and the maintenance of a relatively high degree of antibacterial activity in the crude penicillin produced therein (3), and further study showed that the observed effect was due principally to increased stability of such crude penicillins; the effect was independent of the concentration of the other two principal salts and of the presence (in the concentrations investigated) or absence of phenylacetic acid or of *p*-hydroxy benzoic acid in the culture solution (4). It was also shown that the same results were obtained whether the crude penicillin was produced by *P. notatum* in surface cultures or by *P. chrysogenum* in submerged cultures.

Therefore, it seemed of interest to determine whether a similar action of phosphate could be demonstrated in partially purified preparations of penicillin. The observation that phosphate tends to stabilize penicillin, independently of its buffer action is not without precedent, since it was reported sometime after these experiments were begun that addition of phosphate to dilute aqueous solutions of penicillin may exert a stabilizing effect that is independent of the *p*H value of the solution, and the possible practical application of the observation was pointed out (5). In the present experiments a study was made of the stability of relatively concentrated sterile aqueous solutions of Na penicillin containing different amounts of phosphate and of the stability of sterile lyophilled Na penicillin dried with and without KH_2PO_4. In another set of experiments the stability of sterile aqueous solutions of Na penicillin prepared from material dried with KH_2PO_4 was compared with the stability of similar solutions prepared from material dried without the KH_2PO_4 treatment. Potencies are expressed as Oxford units and were determined by the standard cylinder plate method of assay using *Staph. aureus* NRRL313 (F.D.A. 209P) as the test organism.

EXPERIMENTS AND RESULTS

Aqueous Solutions of Penicillin.—Phosphate buffers (60 parts KH_2PO_4 and 40 parts K_2HPO_4) were

* Received Oct. 15, 1946, from the Research Division, Cutter Laboratories, Berkeley, Calif.

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‡ With the assistance in the laboratory of Ruth Birch, Marjorie Decker, Iola Dunkle, Luba Kellogg, and Patricia Streator Campbell.

diluted with sterile distilled water and sterile aqueous solutions of Na penicillin prepared from regular production lots of dried salt. The initial *p*H of all the preparations, measured by means of a glass electrode, was 6.5–6.6 and did not change more than 0.1 *p*H unit during the period of observation.

In an exploratory experiment of this series (concentration of penicillin = 6240 units/ml.) solutions with phosphate concentrations from 0.001 *M* to 0.04 *M* were investigated.[1] No differences were observed in the half-life[2] of the penicillin solutions stored at 37° until the concentration of phosphate was reduced below 0.005 *M*. Accordingly other experiments were prepared with concentrations of phosphate ranging from 5 millimoles/L. down to zero (distilled water or physiological saline). The *p*H of the penicillin solutions prepared with distilled water was 5.84 and that of the solutions prepared with saline was 5.68. Therefore, in this set of experiments phosphate mixtures with a *p*H of 5.8 were selected. The averaged data from six experiments presented in Figs. 1 and 2 indicate that the phosphate solutions exerted a protective action against

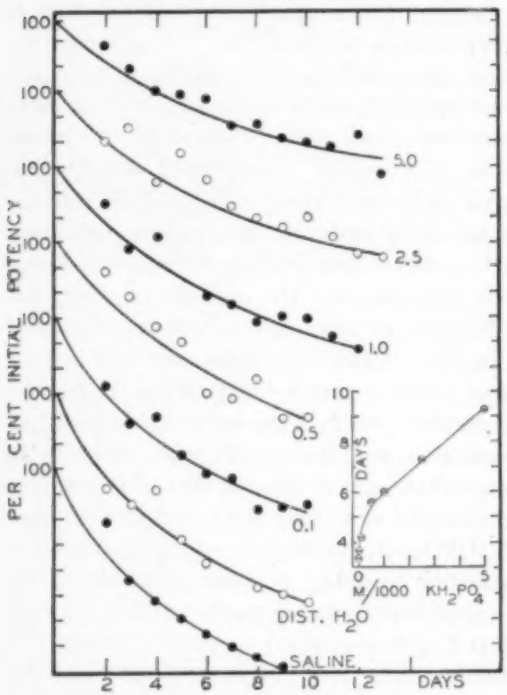

Fig. 1.—Per cent of initial potency remaining in penicillin solutions with different concentrations of phosphate as a function of time at 37°. Figures at the ends of the curves show the concentrations of phosphate in millimoles/L. The ordinate is marked off in intervals of 10%. For convenience the curves are overlapped. *Inset.*—Time required for loss of 50% initial potency as a function of concentration of phosphate.

[1] Na penicillin Cutter, Lot No. 184P1 (purity = 900 units/mg.) was used in this experiment.

[2] Half-life refers to the time required for loss of 50% of the initial potency.

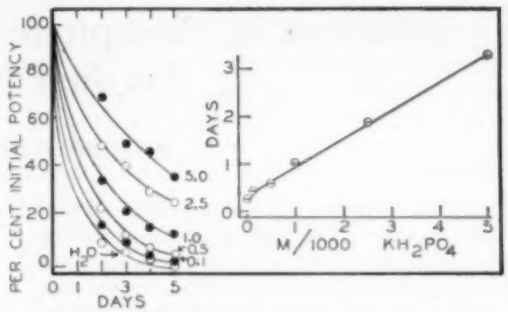

Fig. 2.—Per cent of initial potency remaining in penicillin solutions with different concentrations of phosphate as a function of time at 50°. Figures at ends of curves show concentrations of phosphate in millimoles/L. *Inset.*—Time required for loss of 50% initial potency as a function of concentration of phosphate.

loss of potency of Na penicillin with a relatively high degree of purity. Comparison of these results with those reported previously (4) shows that less phosphate is required to protect partially purified penicillin than the crude material. The absolute rate of loss of potency in aqueous solutions of penicillin was greater in both phosphate and control series, at 50° than at 37°, but it should be pointed out that the relative protection (as shown by prolongation of the half-life) afforded by a given concentration of phosphate was greater at the higher temperature. This is set forth in tabular form below.

TABLE I.—DAYS REQUIRED FOR LOSS OF 50% INITIAL POTENCY IN PENICILLIN SOLUTIONS WITH DIFFERENT CONCENTRATIONS OF PHOSPHATE AND HALF-LIFE OF EACH SOLUTION COMPARED WITH THAT OF CONTROL

Millimoles Phosphate per Liter	Half-Life 37°	Half-Life 50°	Relative Half-Life 37°	Relative Half-Life 50°
5.0	9.50	3.50	2.38	8.75
2.5	7.50	2.00	1.87	5.00
1.0	6.00	1.25	1.50	3.10
0.5	5.50	0.75	1.38	1.88
0.1	4.25	0.50	1.06	1.25
0.0	4.00	0.40	1.00	1.00

A later experiment was designed to study the relation, if any, between the concentration of phosphate/unit of penicillin and the stabilizing effect of phosphates at each of several molar concentrations.[3] Trials were carried out at 50° and at room temperature. Molar concentrations of phosphate ranged from 0 to 0.005 *M* and the concentrations with respect to penicillin ranged from 0 to 0.314 millimole phosphate/10^5 units of penicillin. The initial concentration of penicillin varied from 862 units/ml. to 3560 units/ml. The results of four trials are recorded in Tables II and III and show again the retarding influence of dilute solutions of phosphate on thermal destruction of penicillin and show also that maximum protection of the penicillin under the

[3] Na penicillin Cutter, Lot No. 160P1 (purity = 905 units/mg.) was used in this experiment.

TABLE II.—LOSS OF POTENCY IN PENICILLIN AT 50°

Millimoles Phosphate per Liter	Days Required for Loss 50% Initial Potency at 50° When Millimoles Phosphate/10^5 Units Equal: 0.085 Trial No. 1	2	3	4	Av.	0.184 Trial No. 2	4	Av	0.314 Trial No. 2	4	Av.	0.0 Trial No. 1	2	3	4	Av.
5.0	3.3	3.4	3.3	2.5	3.10	3.8	2.9	3.35	3.1	2.2	2.65	...	...	...	...	...
2.5	1.9	2.7	1.8	1.0	1.85	2.9	1.3	2.10	2.0	1.2	1.60	...	...	...	...	...
1.0	1.1	1.0	1.1	0.4	0.90	2.6	1.3	1.95	2.1	1.0	1.55	...	...	...	...	...
0.5	0.7	0.7	0.6	0.4	0.60	...	0.7	0.70	...	0.6	0.60	...	...	...	...	...
0.1	0.7	0.6	0.5	...	0.60	...	...	...	..	...	...	...	...	...	...	...
0.0 (H_2O)	...	...	...	...	...	...	...	...	..	...	...	0.25	0.3	0.2	0.25	0.25
0.0 (Saline)	...	...	...	...	...	...	...	..	..	...	...	0.30	0.3	0.3	0.30	0.30

TABLE III.—LOSS OF POTENCY IN PENICILLIN AT ROOM TEMPERATURE

Millimoles Phosphate per Liter	Days Required for Loss 25% Initial Potency at Room Temperature When Millimoles Phosphate/10^5 Units Equal: 0.085 Trial No. 1	2	3	4	Av.	0.184 Trial No. 2	4	Av.	0.314 Trial No. 2	4	Av	0.0 Trial No. 1	2	3	4	Av.
5.0	11.2	13.0	12.1	12.5	12.2	19.2	18.0	18.6	12.6	11.8	12.2	...	...	...	...	...
2.5	9.1	12.0	10.0	11.7	10.7	16.4	16.0	16.2	11.4	10.0	10.7	...	...	...	...	...
1.0	9.0	11.0	9.6	14.0	10.9	11.8	10.0	10.9	10.9	10.9	10.9	...	...	...	...	...
0.5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
0.1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
0.0 (H_2O)	...	...	...	...	...	...	...	...	...	...	...	6.0	6.6	6.8	7.0	6.6
0.0 (Saline)	...	...	...	...	...	...	...	...	...	...	...	7.0	6.6	7.0	5.8	6.6

TABLE IV.—DECIMAL FRACTION OF INITIAL POTENCY REMAINING AFTER DIFFERENT LENGTHS OF TIME IN AQUEOUS SOLUTIONS PREPARED FROM Na PENICILLIN DRIED IN THE USUAL WAY (CONTROL) AND DRIED WITH A KH_2PO_4-K_2HPO_4 MIXTURE. (See text for further explanation)

Days of Storage	2° Control	2° Phosphate	25° Control	25° Phosphate	50° Control	50° Phosphate
0	1.00	1.00	1.00	1.00	1.00	1.00
1	..	..	..	..	<0.12	0.19
2	..	..	..	..	$<3.3 \times 10^{-3}$	$<3.3 \times 10^{-3}$
3	..	..	0.51	0.60	2.0×10^{-4}	2.0×10^{-4}
7	1.10	1.12	0.29	0.36	..	..
10	..	..	0.17	0.25	..	..
14	0.79	0.91	0.09	0.14	..	..
17	..	..	0.06	0.07	..	..
21	0.71	0.83	..	..	..	..
25	0.53	0.72	..	..	..	..
32	0.65	0.87	..	..	..	..
39	0.63	0.64	..	..	..	..
46	0.56	0.66	..	..	..	..
53	0.55	0.63	..	..	..	..
66	0.30	0.40	..	..	..	..
74	0.32	0.37	..	..	..	..
81	0.23	0.34	..	..	..	..
88	0.21	0.30	..	..	..	..
95	0.19	0.25	..	..	..	..

conditions of the experiment was afforded by a solution that contained 0.005 *M* phosphate/L. and 0.184 millimole phosphate/10^5 units. Since 10^5 units is the equivalent of 0.168 millimole, it seems that for penicillin of the degree of purity used in the present experiments (ca. 900 units/mg.) maximum protection occurred when the relative concentrations of phosphate and of penicillin in the solution were approximately one to one.

Dried Penicillin.—The following experiments were designed to ascertain whether addition of the requisite amount of phosphate to Na penicillin before final drying would afford a product that possessed greater stability when it was again dissolved preparatory to clinical use and whether the protective action of phosphate could be demonstrated with dried penicillin, and could, therefore, be used to prolong its shelf life. Avoidance of appreciable reduction in purity would be a major concern in any practical application of these results. Therefore, as a compromise in the present set of experiments, concentrations of phosphate were used that were sub-optimal from the standpoint of stabilization of the penicillin. About 10^5 units[4] were dried in the usual manner

[4] Na penicillin Cutter, Lot No. 234P1 was used in this experiment. After drying in the usual way without phosphate, regular production material from this lot had a purity of 1016 units/mg. The moisture content was less than 0.5% (average of two values = 0.25%).

TABLE V.—DECIMAL FRACTION OF INITIAL POTENCY REMAINING AFTER DIFFERENT LENGTHS OF TIME IN VIALS OF Na PENICILLIN (MOISTURE — <0.5%) DRIED IN THE USUAL WAY (CONTROL) AND DRIED WITH A KH_2PO_4-K_2HPO_4 MIXTURE. (See text for further explanation)

Days of Storage	2°		25°		50°	
	Control	Phosphate	Control	Phosphate	Control	Phosphate
0	1.00	1.00	1.00	1.00	1.00	1.00
2	..	..	..	..	..	..
3	..	..	..	..	0.95	1.05
7	..	..	..	..	0.95	1.03
10	..	..	..	..	0.70	0.62
14	..	..	..	..	0.69	0.46
17	..	..	0.96	0.74	..	..
22	..	..	..	..	0.60	0.53
28	..	..	..	..	0.76	0.43
30	0.65	1.03	1.07	0.88	..	..
34	0.99	1.17	1.05	1.13	0.52	0.70
38	..	..	..	..	0.49	0.69
45	0.90	1.13	0.92	1.04	0.70	0.74
58	..	..	0.86	0.97	0.48	0.19
66	..	..	..	..	0.48	0.14
73	..	..	..	..	0.38	0.34
80	0.80	1.03	0.95	0.83	0.28	0.13
87	..	..	..	..	0.22	0.10
94	..	..	..	..	0.22	0.07
101	..	..	..	..	0.19	0.13

(200,000 units/vial). To another aliquot of the same lot was added sufficient KH_2PO_4-K_2HPO_4 mixture to give a phosphate concentration in the final product (after solution as indicated in the next paragraph) of 2.47 millimoles/L. and 0.012 millimole/10^5 units. Then this material was dried in the usual way with the control lot.

After lyophilization the dried material of each vial was dissolved in 10 ml. sterile distilled water and the contents of the several control vials were pooled. Experimental vials were treated similarly. The *p*H of both solutions was 6.5. Then the two solutions were apportioned into two sets of three sterile tubes each and one tube of each was stored at 2°, one was stored at room temperature (ca. 25°) and one was stored at 50°. Samples from the several tubes were assayed at appropriate intervals. The initial potency when the preparations were put in storage was 20,716 units/ml.

The figures in Table IV seem to demonstrate again the stabilizing effect that was noted above, although the magnitude of the difference between the values for control and experimental preparations is not as great as in the earlier experiments. However, it should be pointed out that in this series the molar concentration of phosphate was only about one-half of that found previously to be optimum and that the concentration of phosphate with respect to that of penicillin was less than one-tenth of the optimum.

Another portion of the same lot of penicillin (concentrate = 52,500 units/ml.) was dispensed in individual vials so that each vial received 15,000 units. To a second portion of the concentrate was added a sufficient volume of the phosphate mixture to give 2.47 millimoles phosphate/L. and 0.12 millimole phosphate/10^5 units and it, too, was dispensed into separate vials each containing 15,000 units. Following lyophilization the vials were closed in the usual manner of production lots and each set was divided into three groups which were stored at 2°, room temperature, and 50°, respectively. At appropriate intervals the total contents of vials of each group were assayed.

The results shown in Table V indicate that, in contrast to the situation observed in aqueous solution, the phosphate in the concentrations employed exerted no pronounced stabilizing effect against thermal destruction of penicillin in the dry condition.

SUMMARY

A study was made of the influence of low concentrations of phosphate on the stability of sterile aqueous solutions of Na penicillin and of sterile dry Na penicillin at different temperatures. All preparations in a given series were at the same *p*H.

Low concentrations of phosphate exerted a stabilizing action that was independent of buffering effect on aqueous solutions of penicillin but not on dry penicillin (in the concentrations employed). However, dry penicillin was not damaged by the presence of phosphate and upon subsequent solution was to some extent protected by it from thermal destruction. The concentration of phosphate required to stabilize solutions of partially purified Na penicillin was less than that required to stabilize crude material.

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On the Oxytocic Activity of Pitocin and Pitressin on Isolated Segments of Mammalian Uteri by a Graded Response Method*

By W. M. BACHINSKI and M. G. ALLMARK†

Longitudinal strips of uterine muscle from the guinea pig, rat, rabbit, and human were used for this study. The muscle strips were excised at the normal, parturient, and postpartum states. A marked difference in sensitivity to pitocin and pitressin was observed. The pitocin to pitressin ratios for the guinea pig and rat were quite similar in the normal and postpartum uteri. For the normal rabbit the oxytocic effect of pitressin predominates; in the parturient and postpartum states, however, the oxytocic effect of pitocin was greater. On the parturient human uterus, pitressin exerted an oxytocic effect almost equal to that of pitocin. The oxytocic potency of pitocin expressed in International Units per cc. was found to be about the same on the normal rabbit and parturient human uterine strips but differed from that on the normal guinea pig.

FOLLOWING the work of Kamm, Aldrich, *et al.* (1) the posterior lobe of the pituitary gland was found to contain two active principles, pitocin[1] which stimulates contraction of uterine muscle and pitressin[1] which raises blood pressure. Since then it has been recognized that pitressin possessed little oxytocic activity on the uterus of the virgin guinea pig, using the method of Dale and Laidlaw (2, 3).

Further studies of a qualitative nature in animals using both the intra-uterine hydrostatic bag method and the excised strip technique showed that pitocin and pitressin exert variable effects at the different stages of pregnancy. Furthermore, the effect of pitocin was found to be much greater than that of pitressin at the parturition stage (4–6).

In the human subject, Moir (7) using an intra-uterine hydrostatic bag method found the effect of pitocin to be about three times that of pitressin. More recently Russell (8) has claimed the oxytocic effect of pitressin to be much greater than that of pitocin on human uterine strips excised at the parturient stage of pregnancy. The evidence presented by Russell in support of his statement that pitressin was twenty-five times more potent than pitocin appears rather qualitative and insufficient.

As a result of such divergent views we proceeded to investigate the quantitative relationship between pitocin and pitressin on mammalian uteri using a graded response method (9).

EXPERIMENTAL

The apparatus used in this study and the technique for conducting an assay was the same as that described previously (9, 10). The data were calculated by treating the subdivided muscle strips as separate responses (9), and also by summating the responses for these subdivided muscle strips and then calculating the re-arranged data after the manner of Schild (11). The estimate of potency was the same in both cases with slightly wider limits of error using the second procedure; the results reported are by the latter method.

The magnesium concentration recommended by Van Dyke and Hastings (12) was chosen for the bath solution since this value approximates the serum magnesium level of man as well as of pregnant women (13–15).

The uterine muscle strips were excised at the normal, parturient,[2] and postpartum states. All the human uterine strips were obtained at Caesarean sections performed on patients at or near full term; in each case the uterine muscle was excised from the lower uterine segment. The assays were started within one hour of removal of the strips. In the case of the pregnant animals, longitudinal strips were cut from the uterine tissue covering one or more foeti. The assays on the postpartum animals were all conducted on strips which were excised in most cases from one to three days after parturition.

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[1] Pitocin and pitressin are the trade names given by Parke Davis and Co. to relatively pure extracts of the oxytocic and pressor principles.

[2] By parturient is meant at or shortly before term.

RESULTS

From the data shown in Table I it may be seen that the responses to the various dilutions of pitocin, pitressin, and the Canadian Standard Pituitary Extract used in the assays were quite variable for the different mammalian uteri studied as well as from one assay to another. The guinea pig muscle was found to be the most reactive, the sensitivity being greatest in muscle strips excised from normal and parturient guinea pigs. The sensitivity of the remaining mammals as compared to the guinea pig was in the following order of decreasing response: rat, rabbit, and human. There was an increase in sensitivity in the rabbit during the pregnant and postpartum stages for both hormones. The range of sensitivity of the human uterine muscle strips was found to be about the same as for the parturient rabbit.

The oxytocic activity of pitocin in terms of pitressin as determined by assay on various mammalian uteri is shown in Table II. The oxytocic effects shown for the normal and postpartum guinea pig and for the normal and postpartum rat were quite similar, but the effects on the parturient uterus of the guinea pig and the rat were quite different; the oxytocic activity of pitocin in the parturient rat was about twice that found in the guinea pig. In the case of uterine muscle from the normal rabbit, however, the effect of pitressin was found to be almost twice that of pitocin. The relative oxytocic activity of pitocin increased during pregnancy and at the postpartum state reached a peak of 5 to 1. In the parturient human uterine muscle the pitocin to pitressin ratio was almost 1:1. These potency ratios are shown graphically in Fig. 1. The differences between these ratios were analyzed statistically (16), and it may be seen from Table III that they were

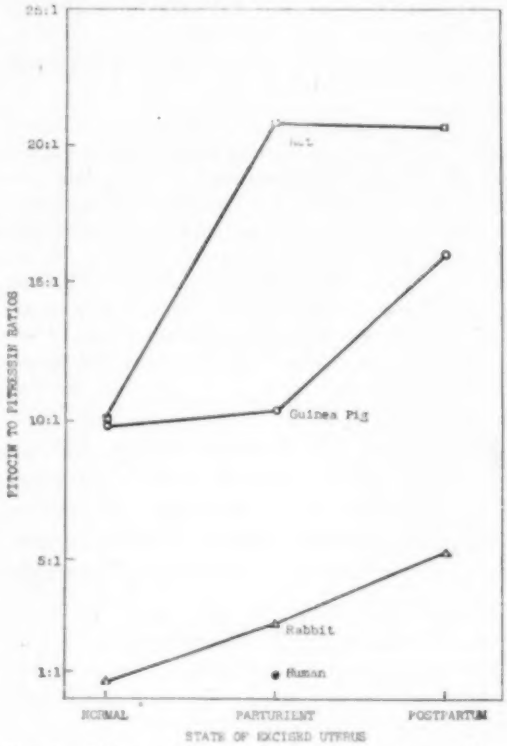

Fig. 1.—Pitocin to pitressin ratios on mammalian uteri.

TABLE I.—DILUTIONS OF PITOCIN, PITRESSIN, AND THE CANADIAN STANDARD PITUITARY EXTRACT USED IN THE ASSAYS[a]

Source of Uterine Muscle	Normal Uterus	Uterus at the Peak of Pregnancy	Postpartum Uterus
Guinea pig	Pitocin, 1:200 to 1:500 Pitressin, 1:16 to 1:32 Can. Std., 1:50 to 1:100	Pitocin, 1:100 to 1:400 Pitressin, 1:4 to 1:50	Pitocin, 1:50 to 1:100 Pitressin, 1:2.5 to 1:5
Rat	Pitocin, 1:40 to 1:100 Pitressin, 1:10 to 1:40	Pitocin, 1:50 to 1:100 Pitressin, 1:5	Pitocin, 1:20 Pitressin, 1:5
Rabbit	Pitocin, 1:1 to 1:10 Pitressin, 1:1 to 1:10 Can. Std., 1:1 to 1:10	Pitocin, 1:10 to 1:50 Pitressin, 1:10 to 1:50	Pitocin, 1:25 to 1:50 Pitressin, 1:6 to 1:12
Human	.....	Pitocin, 1:2 to 1:45 Pitressin, 1:2 to 1:45 Can. Std., 1:1	.....

[a] These dilutions were used in a bath solution of 550 cc. capacity.

TABLE II.—COMPARISON OF PITOCIN TO PITRESSIN RATIOS OBTAINED ON VARIOUS MAMMALIAN UTERIA[a]

Source of Uterine Muscle	Normal Uterus		Uterus at Peak of Pregnancy		Postpartum Uterus	
	Pitocin to Pitressin Ratio	Limits for 95 Times Out of 100	Pitocin to Pitressin Ratio	Limits for 95 Times Out of 100	Pitocin to Pitressin Ratio	Limits for 95 Times Out of 100
Guinea pig	9.8 to 1.0	9.4 to 10.2	10.8 to 1.0	8.5 to 13.8	17.0 to 1.0	14.4 to 20.0
Rat	10.1 to 1.0	8.9 to 11.4	21.8 to 1.0	18.5 to 25.8	21.3 to 1.0	16.9 to 26.9
Rabbit	0.6 to 1.0	0.5 to 0.8	2.6 to 1.0	2.3 to 2.9	5.3 to 1.0	4.3 to 6.4
Human	.....	.....	0.9 to 1.0	0.7 to 1.0	.....	.....

[a] Each ratio is the weighted mean of two or more assays.

all significantly different from the corresponding guinea pig results except in the case of the normal and postpartum rat. The potency ratios of uterine muscle strips from the parturient human and the normal rabbit were not significantly different.

TABLE III.—SIGNIFICANCE OF PITOCIN TO PITRESSIN RATIOS OBTAINED ON VARIOUS MAMMALIAN UTERI, USING THE GUINEA PIG AS A BASIS FOR COMPARISON[a]

Source of Uterine Muscle	Normal Uterus	Uterus at Peak of Pregnancy	Postpartum Uterus
Guinea pig	...	...	...
Rat	NS	S	NS
Rabbit	S	S	S
Human	...	S	...

[a] S = significant.
NS = not significant.

These differences in pitocin to pitressin ratios suggested that pitocin might not possess the labeled potency in International Units when assayed on mammalian uteri other than the guinea pig. Accordingly, this product was assayed against the Canadian Standard Pituitary Extract using uterine muscle strips taken from the guinea pig, rabbit, and human subject. The results are shown in Table IV. The rabbit and human assay results are significantly different from the guinea pig result. The normal rabbit and the parturient human assay results are similar. The unitage found for the respective uteri was as follows: guinea pig, 9.5 I. U./cc.; rabbit, 6.2 I. U./cc.; human, 7.4 I. U./cc.

Kamm (17), who stated that in his laboratory pitocin and pitressin were equally active on the parturient uterus *in vitro*. These results, however, do not agree with those of Russell (8) who reported that pitressin was approximately twenty-five times more potent a stimulant for the human parturient uterus than pitocin. Furthermore, the results do not agree with clinical findings as reported by Moir (7). In this respect it may be stated that the intra-uterine bag method generally used in clinical work has several disadvantages reported by Robertson (18) and Adair and Haugen (19), probably the most important being the emotional factor which in itself may produce contractions.

One of the explanations advanced for these differences in the oxytocic response of mammalian uteri to pitocin and pitressin are the effects of certain hormones present during the normal and gestational periods. It has been demonstrated in previously published studies that progestin and gonadotrophic hormones produced no desensitization of the reactivity to oxytocin or inhibition of the motility of the uterus *in vivo* or *in vitro* in the guinea pig. In the mouse the response of the uterus to oxytocin is greatly increased by the administration of oestrin but cannot be counteracted by progestin

TABLE IV.—THE POTENCY OF PITOCIN IN INTERNATIONAL UNITS WHEN ASSAYED ON VARIOUS MAMMALIAN UTERI[a]

Source of Uterine Muscle	Found Potency in I. U./Cc.	Actual Limits in I. U./Cc. for 95 Times Out of 100	Per Cent of Guinea Pig Result	Result Significantly or Not Significantly Different from the Guinea Pig
Normal guinea pig	9.5	8.7 to 10.4	..	...
Normal rabbit	6.2	5.6 to 6.8	65.3	S
Parturient human	7.4	6.8 to 8.1	77.9	S

[a] Results are the weighted means of two or more assays.

DISCUSSION

On the basis of these results it would appear that the oxytocic response of the normal rabbit uterus to pitocin and pitressin approximates most closely that of the parturient human, both in the cases of the potency ratios of pitocin to pitressin, as well as in the unitage assigned to pitocin when assayed against the Canadian Standard Pituitary Extract. The oxytocic activity of pitocin in terms of pitressin on the parturient human uterus *in vitro* agrees with the findings of which in the rabbit inhibits the response (6).

If the response of the parturient human uterus *in vitro* to purified extracts from the posterior pituitary gland is to be taken as the basis of comparison, then the present pharmacopœial methods do not measure the true oxytocic activity of these products.

The evidence presented suggests further that any indirect method such as the chicken blood pressure depressor method (15) may not agree with a method using rabbit or human uterine muscle strips.

SUMMARY AND CONCLUSIONS

1. The oxytocic response to pitocin and pitressin of uterine muscle strips from the normal, parturient, and postpartum guinea pig, rat, rabbit, and parturient human has been compared.

2. The sensitivity of rabbit and human uterine muscle strips to pitocin and pitressin was found to be about the same. The order of decreasing sensitivity for the uterine muscle strips was as follows: guinea pig, rat, rabbit, and human.

3. The oxytocic activity of pitressin was almost equal to that of pitocin on parturient human uterine strips excised from the lower segment.

4. The pitocin to pitressin ratios for the mammalian uteri were approximately as follows: normal guinea pig and rat, 10 to 1; parturient and postpartum rat and postpartum guinea pig, 20 to 1; normal rabbit, 0.6 to 1; parturient and postpartum rabbit, 2.6 to 1 and 5.3 to 1, respectively.

5. The oxytocic activity of pitocin was found to be about the same on the normal rabbit and parturient human uterine strips but differed significantly from the activity on the normal guinea pig.

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Synthesis of Iodosulfophenylazo and Iodocarboxyphenylazo Derivatives of 1-Amino-8-Naphthol-2,4-Disulfonic Acid*

By HAAKON BANG†

Trisodium salts of (2,6-diiodo-4-sulfophenylazo) - 1 - amino - 8 - naphthol - 2,4-disulfonic acid and of (2,6-diiodo-4-carboxyphenylazo) - 1 - amino - 8 - naphthol - 2,4-disulfonic acid have been prepared.

THE PRESENT report on the iodination of azo compounds is a continuation of the one made by Klemme and Bang (1). The purpose in making this study was to produce radiographic opaque agents which would be still more soluble than those previously prepared. The two compounds reported herein are to be used in pharmacological and radiological studies which will follow later.

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EXPERIMENTAL

3,5-Diiodosulfanilic Acid.—This compound was prepared by a modification of the method first described by Boyle (2), and by a method reported by Klemme and Bang (1).

The Trisodium Salt of (2,6-Diiodo-4-Sulfophenylazo)-1-Amino-8-Naphthol-2,4-Disulfonic Acid.—This dye was prepared by suspending 21 Gm. of 3,5-

diiodosulfanilic acid in 92 cc. of 10% hydrochloric acid and the mixture cooled in an ice bath to 0° with constant stirring. Three and five-tenths grams of sodium nitrite, dissolved in 4 cc. of cold distilled water, was added, a small portion at a time. After the last addition, the mixture was tested with starch iodide test paper. A small quantity of the diiodosulfanilic acid was added until the test for free nitrous acid was just negative.

The cold mixture containing the 3,5-diiodo-4-diazobenzene-1-sulfonic acid was poured, a small portion at a time, into a solution containing 16 Gm. of 1-amino-8-naphthol-2,4-disulfonic acid and 15 Gm. of sodium hydroxide. The resulting red solution was heated to boiling and finally evaporated to dryness on a water bath. The residue was powdered and extracted with 500 cc. of absolute methanol.

The methanol extract was filtered and evaporated to dryness and the resulting red residue ground to a fine powder in a mortar. The dye was then washed with a solution of three parts of ether and one part of ethanol and dried in an oven at 105°. It was then analyzed for its iodine content by the method used by Klemme and Hunter (3).

Anal.—Calcd. for $C_{16}H_8I_2N_2Na_2O_{10}S_2$: I, 30.9%. Found: I, 31.4% and 31.3%.

The dye is a dark red powder, insoluble in ether, chloroform, and acetone. It is soluble in ethanol and methanol. One gram is soluble in about 4.8 Gm. of water.

The yield was 28.6%.

3,5-Diiodo-4-Aminobenzoic Acid.—This compound was first synthesized by Michael and Norton (4). It was prepared in this study by the method described by Klemme and Bang (1).

(2,6 - Diiodo - 4 - Carboxyphenylazo) - 1 - Amino-8-Naphthol-2,4-Disulfonic Acid.—Nineteen and four-tenths grams of 3,5-diiodo-4-aminobenzoic acid was dissolved in 75 cc. of concentrated sulfuric acid, placed in an ice bath and cooled to 0°. Three and five-tenths grams of sodium nitrite was added in small portions until starch iodide test paper indicated a faint positive test for nitrous acid. A small portion of the 3,5-diiodo-4-aminobenzoic acid was added until the test was just negative. The cold diazonium mixture was carefully poured into a liter beaker almost filled with cracked ice.

Sixteen grams of 1-amino-8-naphthol-2,4-disulfonic acid was dissolved in a liter of distilled water containing 100 Gm. of sodium hydroxide. The diluted diazonium mixture was poured slowly and with constant stirring into the naphthol solution. Care was taken that the coupling solution was always alkaline.

The resulting red solution was heated to boiling, allowed to cool, and finally made acid with hydrochloric acid to precipitate the dye. The dye was then thrown down by centrifugation and the supernatant liquid drawn off. The dye was resuspended in 10% hydrochloric acid and again centrifuged. This process was repeated. The precipitate was collected and dried in an oven at 105°. The acid was analyzed as before for its iodine content.

Anal.—Calcd. iodine for $C_{17}H_{11}I_2N_3O_8S_2$: I, 35.3%. Found: I, 34.6% and 34.6%.

The trisodium salt was formed by dissolving 7.5 Gm. of the dye and 1.6 Gm. analytical reagent sodium hydroxide in approximately 15 cc. of distilled water. The solution was evaporated to dryness and dissolved in 100 cc. of absolute methanol. The trisodium salt of (2,6-diiodo-4-carboxyphenylazo)-1-amino-8-naphthol-2,4-disulfonic acid is a red powder which is soluble in methanol and ethanol. It is insoluble in chloroform, ether, and acetone. One gram is soluble in about 5.15 Gm. of water.

The yield was 44.5%.

SUMMARY

The trisodium salts of (2,6-diiodo-4-sulfophenylazo)-8-amino-1-naphthol-2,6-disulfonic acid and 2,6-diiodo-4-carboxyphenylazo)-1-amino-8-naphthol-2,4-disulfonic acid were prepared. The dyes were analyzed for their iodine content and their solubilities determined.

The position of attachment of the phenylazo group on the naphthol ring was not determined. Pharmacological and radiological tests will follow later.

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The *In Vitro* Antibacterial Action of Dialkylaminoalkyl Ethers of Stilbestrol and Hexylresorcinol*

By C. W. CHAPMAN, G. P. HAGER, and D. E. SHAY

Directions are given for the preparation of stilbestryl-bis(diethylaminoethyl) ether dihydrochloride, and hexylresorcinol-bis (diethylaminoethyl) ether. *In vitro* tests showed that the stilbestrol derivative is bactericidal against Mycobacterium tuberculosis var. hominis in concentration of 1:50,000; against cultures of the M. tuberculosis which had been pretreated with alkali, 1:50,000; against Eberthella typhosa, 1:5000 and against Staphylococcus aureus, 1:5000. The corresponding minimum effective concentrations of the hexylresorcinol derivative were 1:50,000 (M. tuberculosis); 1:100,000 (M. tuberculosis pretreated with alkali); 1:50,000 (E. typhosa; and 1:10,000 (S. aureus). The compounds appeared to be bactericidal rather than bacteriostatic against all three organisms. Preliminary tests indicated that the stilbestrol derivative, administered intraperitoneally to castrated female rats, was neither estrogenic nor toxic in doses of 10, 50, or 100 micrograms.

IT HAS BEEN found that certain dialkylaminoalkyl ethers of halogen-substituted phenols possess tuberculostatic activity when tested *in vitro* and *in vivo* (1, 2). This type of activity was not observed in nonhalogenated dialkylaminoalkyl phenyl ethers (2). Since stilbestrol, a nonhalogenated phenol, has been shown to be bactericidal to tubercule bacilli in dilutions of 1:5000 *in vitro* (3), it was of interest to study the antitubercular activity of its dialkylaminoalkyl ethers. Also included in this study is a dialkylaminoalkyl ether of hexylresorcinol.

To make possible a comparison with work previously reported (1–3), it was decided to use two "treated" and two untreated samples of sputum containing Mycobacterium tuberculosis var. hominis. Four types of selective media were employed to determine possible effects of media on the antibacterial tests. Methods of inoculation and periods of incubation were selected that were considered most suitable for a test of antibacterial agents on the tubercle bacilli *in vitro*.

In order to obtain a more comprehensive picture of the antibacterial activity of the phenolic ethers, antibacterial tests were carried out using Eberthella typhosa and Staphylococcus aureus as the test organisms.

Since *in vivo* tests are contemplated, a preliminary investigation of the stilbestrol derivative (stilbestryl-bis(diethylaminoethyl) ether dihydrochloride) for estrogenic activity has been carried out.

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Presented to the Scientific Section, A. PH. A., Pittsburgh meeting, 1946.

EXPERIMENTAL

The bis(diethylaminoethyl) ethers of stilbestrol and hexylresorcinol were prepared in the usual way. Diethylaminoethyl chloride hydrochloride was prepared from 2-diethylaminoethanol by reaction with thionyl chloride in dry benzene (2).

Stilbestryl-bis(diethylaminoethyl) Ether.—Stilbestrol (27 Gm.; 0.1 mol.) was dissolved in a solution of sodium (9.2 Gm.; 0.4 equivalent) in methanol (250 cc.). Diethylaminoethyl chloride hydrochloride (42 Gm.; 0.25 mol.) was added, and the resulting solution was stirred under reflux on a steam bath for ten hours. After filtering off the NaCl, the solvent was removed by distillation at reduced pressure. The residue was taken up in water and ether, and the aqueous layer was extracted thoroughly with ether. The ether solution was washed with 20% NaOH and then with water to remove unchanged stilbestrol, dried over anhydrous Na_2SO_4, and concentrated by distillation of the ether. The light tan solid residue was taken up in alcohol saturated with HCl. After addition of dry ether, the white crystalline dihydrochloride separated and was purified by crystallization from an ethanol-ethyl acetate mixture (1:1).

Stilbestryl-bis(diethylaminoethyl) ether dihydrochloride, thus obtained, melted at 236–237° with slight decomposition.

Anal.—Calcd. for $C_{30}H_{48}N_2O_2Cl_2$: N, 5.19. Found: 5.26.

The bis-quaternary ammonium salt was prepared from the free base with methyl iodide in ether at room temperature. The amorphous solid which separated was recrystallized from ethanol and almost colorless flat needles, m. p. 241–242°, were obtained.

Anal.—Calcd. for $C_{32}H_{52}N_2O_2I_2$: N, 3.73. Found: N, 3.90.

Hexylresorcinol-bis(diethylaminoethyl) Ether.—Hexylresorcinol[1] (19.5 Gm.; 0.1 mol.) was dissolved in a solution of sodium (4.6 Gm.; 0.2 equivalent) in methanol (200 cc.). Diethylaminoethyl chloride hydrochloride (20.7 Gm.; 0.12 mol.) was added and the solution was refluxed with stirring for ten hours. The NaCl was filtered off and the alcohol removed by distillation at reduced pressure. The residual red oil was taken up in ether and extracted with dilute HCl to separate the amino ethers from unchanged hexylresorcinol. The aqueous layer was neutralized with $NaHCO_3$ and extracted with ether. The ethereal extract was dried over anhydrous Na_2SO_4; and, after removal of the solvent, the product was fractionally distilled. The fraction boiling at 214–222°/8 mm. was collected and fractionated again, and the product which boiled at 220–225°/11 mm. was obtained. Nitrogen analysis of this thick yellow oil which darkened on standing, indicated that it was principally the di-ether, contaminated with appreciable amounts of the mono-ether. Efforts to separate the mixture through formation of a soluble sodium derivative of the mono-ether were unavailing.

The hydrochloride, phosphate, perchlorate, and quaternary salts (with methyl iodide or benzyl chloride) were obtained as oils. The dipicrate of hexylresorcinol-bis(diethylaminoethyl) ether was prepared in the usual way and found to melt at 108–109.5° after repeated crystallization from ethanol.

Anal.—Calcd. for $C_{36}H_{58}N_8O_{14}$: N, 13.18. Found: 13.14.

Formation of the picrate of the di-ether in good yield confirms previous evidence that the basic oil consists chiefly of hexylresorcinol-bis(diethylaminoethyl) ether.

Bacteriological

The *p*H of freshly prepared solutions of stilbestrylbis(diethylaminoethyl) ether and hexylresorcinol-bis (diethylaminoethyl) ether in 0.3 *N* HCl was adjusted to 7 with 0.1 *N* NaOH and the solutions were diluted to 50 cc. in a volumetric flask with distilled water (final concentration, 1:100). The action of various dilutions of the stock solutions thus prepared on the tubercle bacilli will be considered first.

Cultures.—Four cultures of positive sputum (A, B, C, D)[2] containing Mycobacterium tuberculosis var. hominis were used in the experiment. Cultures A and B were untreated while cultures C and D were treated with equal volumes of 4% NaOH, placed in sterile centrifuge tubes, and incubated at 37° for thirty minutes. These cultures were next centrifuged for thirty minutes at 3600 r. p. m. and the supernatant fluid discarded. The sediment was treated with sterile 2.5 *N* HCl and neutralized with 0.1 *N* NaOH using bromthymol-blue indicator.

Media.—Since cultures A and B were untreated, it was thought that the contaminating organic matter might inhibit or reduce the activity of the stilbestrol and hexylresorcinol derivatives. After the cultures had been prepared as stated above, each was inoculated into tubes of Petragnani's medium in order to secure pure cultures.

Glycerol broth, Long's synthetic medium, glycerol agar, and Dorset egg medium were selected as the experimental culture media. The first three of these media were adjusted to *p*H 7 with 0.1 *N* NaOH, cooled to 50°, and treated aseptically with sufficient amounts of the stock solutions of the stilbestrol and hexylresorcinol derivatives (previously sterilized by filtration) to give concentrations from 1:500 to 1:200,000. The Dorset egg medium was prepared in the usual way. The various amounts of the sterile stock solutions of the stilbestrol and hexylresorcinol derivatives were added before the medium was inspissated for two hours at 70–75° on three successive days.

Each series of tubes containing the experimental media with varying concentrations of the antibacterial agents were inoculated with one of the four different cultures of the tubercle bacilli and plugged with sterile rubber stoppers. The cultures were incubated in a slanting position in the dark at a constant temperature of 37.5°.

After incubation of the cultures for seven days, they were examined for growth of the tubercle bacilli. The tubes containing Dorset egg medium were examined for the typical grayish yellow growth which is present when tubercle bacilli grow on this medium. The tubes containing the glycerol agar were examined for the presence of the cream-colored raised colonies which are characteristic of tubercle bacilli grown on this medium. When no growth was in evidence, the tubes were again placed in the incubator for another seven days. In those tubes containing Long's synthetic medium and glycerol broth, growth of the tubercle bacilli was not determined by inspection as above. Instead, material from these tubes was inoculated into Petragnani's medium and permitted to incubate for six weeks at 37.5° at which time they were examined for growth. Similar examinations of all the experimental media were made on the 14th, 21st, 28th, 35th, 42nd, and 90th days of incubation. As growth appeared on the glycerol agar, Petragnani's, and Dorset egg media, results were recorded as positive and were indicated by means of a plus sign in Tables I and II. After ninety days of incubation of the experimental media, Kinyoun's carbol-fuchsin acid-fast stain was used to confirm the presence of tubercle bacilli.

The relative antitubercular activities of the stilbestrol and hexylresorcinol derivatives, determined by employing a wide range of decimal dilutions in the experimental media, are indicated in Tables I and II. The hexylresorcinol derivative was bactericidal against treated tubercle bacilli in concentration of 1:100,000, whereas the stilbestrol derivative was bactericidal in concentration of 1:50,000. Cultures

[1] Hexylresorcinol was obtained through the courtesy of Dr. J. M. Sprague, Sharp and Dohme, Glenolden, Pa.

[2] Positive cultures of sputum used in this experiment were obtained from the Maryland State Health Department.

TABLE I.—THE ANTIBACTERIAL ACTIVITY OF STILBESTRYL-BIS(DIETHYLAMINOETHYL)ETHER ON MYCOBACTERIUM TUBERCULOSIS VAR. HOMINIS[a]

Dilutions[b] in 1000's	Sputum Samples	Media	Results of Incubating for (Days) X[c]	7	14	21	28	35	42	90	Petragnani's Medium 6 Weeks' Incubation	Kinyoun's Carbol-Fuchsin Acid-Fast Stain
10	A	4	+	−	−	−	−	−	−	−	−	−
	B	3	+	−	−	−	−	−	−	−	−	−
	C	2	+	−	−	−	−	−	−	−	−	−
	D	1	+	−	−	−	−	−	−	−	−	−
50	A	1	+	−	−	−	−	−	−	−	−	−
	B	2	+	−	−	−	−	−	−	−	−	−
	C	3	+	−	−	−	−	−	−	−	−	−
	D	4	+	−	−	−	−	−	−	−	−	−
100	A	1	+	−	−	+	+	+	+	+	+	+
	B	2	+	−	−	−	−	+	+	+	+	+
	C	3	+	−	−	+	+	+	+	+	+	+
	D	4	+	−	−	−	+	+	+	+	+	+
200	A	1	+	−	−	−	−	+	+	+	+	+
	B	2	+	−	−	−	−	−	+	+	+	+
	C	3	+	−	−	−	+	+	+	+	+	+
	D	4	+	−	−	+	+	+	+	+	+	+

[a] Key: A and B—Untreated sputum.
C and D—Treated sputum.
1—Long's synthetic medium.
2—Glycerol broth.
3—Glycerol agar.
4—Dorset egg medium.
[b] Dilutions of 0.5 and 1 are not included above.
[c] Control tubes of Petragnani's medium free of Stilbestrol derivative.

TABLE II.—THE ANTIBACTERIAL ACTIVITY OF HEXYLRESORCINOL-BIS(DIETHYLAMINOETHYL) ETHER ON MYCOBACTERIUM TUBERCULOSIS VAR. HOMINIS[a]

Dilutions[b] in 1000's	Sputum Samples	Media	Results of Incubating for (Days) X	7	14	21	28	35	42	90	Petragnani's Medium 6 Weeks' Incubation	Kinyoun's Carbol-Fuchsin Acid-Fast Stain
10	A	3	+	−	−	−	−	−	−	−	−	−
	B	2	+	−	−	−	−	−	−	−	−	−
	C	3	+	−	−	−	−	−	−	−	−	−
	D	2	+	−	−	−	−	−	−	−	−	−
50	A	2	+	−	−	−	−	−	−	−	−	−
	B	3	+	−	−	−	−	−	−	−	−	−
	C	2	+	−	−	−	−	−	−	−	−	−
	D	3	+	−	−	−	−	−	−	−	−	−
100	A	3	+	−	−	−	+	+	+	+	+	+
	B	2	+	−	−	−	−	−	+	+	+	+
	C	3	+	−	−	−	−	−	−	−	−	−
	D	2	+	−	−	−	−	−	−	−	−	−
200	A	3	+	−	−	−	−	+	+	+	+	+
	B	2	+	−	−	−	−	+	+	+	+	+
	C	2	+	−	−	−	+	+	+	+	+	+
	D	3	+	−	−	−	+	+	+	+	+	+

[a] Key: A and B—Untreated sputum.
C and D—Treated sputum.
X—Control tubes of Petragnani's medium free of hexylresorcinol derivative.
2—Glycerol broth.
3—Glycerol agar.
[b] Dilutions of 0.5 and 1 are not included above.

TABLE III.—THE BACTERICIDAL ACTIVITY OF HEXYLRESORCINOL-BIS(DIETHYLAMINOETHYL) ETHER AND STILBESTRYL-BIS(DIETHYLAMINOETHYL) ETHER ON STAPHYLOCOCCUS AUREUS AND EBERTHELLA TYPHOSA[a, b]

Dilutions in 1000's	Hexylresorcinol-bis(diethylaminoethyl) Ether — Staphylococcus aureus — Time Intervals in Minutes						Hexylresorcinol-bis(diethylaminoethyl) Ether — Eberthella typhosa — Time Intervals in Minutes						Stilbestryl-bis(diethylaminoethyl) Ether — Staphylococcus aureus — Time Intervals in Minutes						Stilbestryl-bis(diethylaminoethyl) Ether — Eberthella typhosa — Time Intervals in Minutes					
	5	10	15	30	60	90	5	10	15	30	60	90	5	10	15	30	60	90	5	10	15	30	60	90
1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	+	−	−	−	−	−
10	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
50	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] Controls-sterility tests were conducted on all experimental media.
[b] To avoid duplication, results of subculturing of all nutrient broth tubes on nutrient agar plates have been omitted from the above data.

of the tubercle bacilli that had been treated with alkali had a lower resistance to the antibacterial agents than untreated cultures. It has been suggested (3) that the lowered resistance of cultures of this nature is due to the action of the NaOH which has two effects: the breaking-up of clumps of bacteria and the digestion of certain organic materials which usually surround the organism. This action affords the antibacterial agent a greater opportunity to act on the organism.

The antibacterial activity of both the stilbestrol derivative and the hexylresorcinol derivative on untreated samples of sputum containing the tubercle bacilli was indicated by absence of growth in culture tubes containing the agents in concentration of 1:50,000. As indicated in Table I, it was not possible to note any difference in the activity of the stilbestrol derivative on treated and untreated samples of sputum because of the range of the decimal dilutions employed. The rate of growth of the samples (A, B, C, D) in presence of 1:100,000 concentration of the stilbestrol derivative was approximately the same, with growth appearing on all the experimental media between three and four weeks after inoculation.

In order to determine whether the antibacterial agents in the concentrations employed were bacteriostatic or bactericidal, cultures whose growth had been completely inhibited by the compounds during incubation were subcultured on fresh Petragnani's medium as a control. In Tables I and II, the results of subculturing on the control media are summarized. Subcultures on Petragnani's medium, after incubation, showed either the presence of typical colonies or complete absence of growth. From results of this type, it is suggested that the stilbestrol and hexylresorcinol derivatives have little or no bacteriostatic activity on tubercle bacilli under these experimental conditions, but that they do have a pronounced bacteriocidal action at a sufficiently high concentration.

Long's synthetic medium, glycerol broth, glycerol agar, and Dorset egg medium were selected as the experimental media because they represent the more common types of specific media used for the growth of tubercle bacilli, and because they afford an opportunity to observe whether the type of medium has an influence on the rate or amount of growth of treated and untreated cultures. In Table I, all tubes containing the stilbestrol derivative in concentration of 1:100,000 were the first to show that growth was not inhibited by the compound. Growth appeared in the four different types of experimental media in the following sequence: Long's synthetic medium and glycerol agar, between fourteen and twenty-one days; Dorset's egg medium, between twenty-one and twenty-eight days and glycerol broth, between twenty-eight and thirty-five days. Verification of growth was accomplished by examination of the solid media for typical colonies and staining smears from the liquid media by means of Kinyoun's carbol-fuchsin acid-fast stain. Growth in control tubes of identical media used in the experiment appeared in approximately the same interval of time after incubation was begun. Thus it is believed that the type of medium, containing a sublethal concentration of the antibacterial agent, had no influence on the growth of the organism. Since results of this kind were obtained in experiments on the stilbestrol derivative, the experimental culture media used in testing the antitubercular activity of the hexylresorcinol derivative were limited to glycerol broth and glycerol agar.

Staphylococcus aureus and Eberthella typhosa.—Further studies were conducted in an attempt to ascertain the extent of the antibacterial action of the two compounds on Staphylococcus aureus (Strain 209) and Eberthella typhosa standard culture for use in phenol coefficient tests (Table III).

Nutrient broth was selected as the culture medium. It was prepared in the usual way, placed in test tubes, and sterilized at 15 pounds pressure (121°) for fifteen minutes; *p*H 6.8. Dilutions of the antibacterial agents were made by the method previously described. To a series of tubes containing 5 cc. of culture medium containing the desired concentration of the compound, 0.5 cc. of a twenty-four hour broth culture of Staphylococcus aureus was added at intervals of thirty seconds. From this point the procedure followed was that recommended by the Food and Drug Administration for testing the bactericidal properties of antiseptics and disinfectants except that the tubes were subcultured after thirty, sixty, and ninety minutes in addition to the prescribed five-, ten-, and fifteen-minute intervals (4). Similar procedures were followed using Eberthella typhosa. The tubes were incubated for forty-eight hours at 37° and then subcultured on nutrient agar plates.

Subculturing was performed on all tubes of nutrient broth whether or not they showed any turbidity, making it possible to determine the activity of the compounds as bacteriostatic or bactericidal agents. The results were similar to those found when using the tubercle bacillus as the test organism. In all tubes in which the experimental medium was free of turbidity, subculturing on nutrient agar plates showed the complete absence of growth. To avoid duplication, results of subculturing on nutrient agar plates were omitted from Table III. It was possible by subculturing to identify the organism by means of the growth characteristics and by staining.

The hexylresorcinol derivative proved to be a more efficient antibacterial agent than the stilbestrol derivative, since it killed Staphylococcus aureus in concentration of 1:10,000 after being in contact with the organism for sixty minutes and Eberthella typhosa in concentration of 1:50,000 after being in contact with the organism for ninety minutes. The stilbestrol derivative killed Staphylococcus aureus and Eberthella typhosa in concentration of 1:5000 after exposures of sixty minutes and ten minutes, respectively. Both of the compounds were more ac-

tive against Eberthella typhosa than against Staphylococcus aureus.

Pharmacological

The vaginal smear method employing castrated adult female white rats was used in the study of the stilbestrol derivative for estrogenic activity. In preliminary experiments, doses of 10, 50, and 100 micrograms, administered intraperitoneally three times daily for three days, failed to elicit any positive responses. Stilbestrol in the same series of rats in doses of 1, 5, and 10 micrograms gave marked positive reactions in all the animals. These tests, using a limited number of animals, suggest that the stilbestrol derivative is devoid of estrogenic activity. Furthermore no toxic reactions from the stilbestrol derivative were observed in any of the animals at the dose levels administered.

SUMMARY

1. The preparation of stilbestryl-bis(diethylaminoethyl) ether and hexylresorcinol-bis(diethylaminoethyl) ether has been described.

2. The stilbestrol and hexylresorcinol derivatives had a marked antibacterial action on the tubercle bacilli *in vitro*, were less active against Eberthella typhosa, and still less active against Staphylococcus aureus. The hexylresorcinol derivative was more active than the stilbestrol derivative against all organisms tested.

3. Preliminary tests indicated that the stilbestrol derivative had no estrogenic activity in doses that were 100 times the effective dose of stilbestrol.

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On the Stability of Vitamin C in Aqueous Solutions and in Pharmaceutical Preparations*,†

By FRANCISCO GIRAL‡

The effects of several factors such as dilution, alcohol, metals, *p*H, and concentration have been determined. The results are reported in terms of the number of days required for 50 per cent destruction of vitamin C under various conditions.

THE GREAT readiness with which vitamin C is destroyed in aqueous solutions requires special precautions in its pharmaceutical preparation. A practically permanent stability in injections is obtained with relative ease, but it is more difficult in syrups or elixirs, with large volumes of liquids, and with receptacles not hermetically sealed. Being indefinitely stable in solid form, there exists one possibility for guaranteeing the stability: to dispense the solid vitamin C, alone or with other solid components, in a separate receptacle and to mix it with the syrup or elixir at the time of initiating its use. Because a bottle of this type will be consumed in from five to ten days, the problem of the stability is reduced to that period of time.

Though the factors which accelerate or retard the oxidative destruction of vitamin C are well known, the bibliographical information is insufficient to resolve the pharmaceutical problem of the stability in terms of days.

EXPERIMENTAL

The determinations were made with 2,6-dichlorophenolindophenol. The solutions were kept in 250-cc. amber flasks, at room temperature, with cork

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TABLE I.—EFFECT OF CONCENTRATION ON THE RATE OF DESTRUCTION OF VITAMIN C

	Days																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	20	25	30
I—Vitamin C alone																		
I-A = 200 mg. % water A	90.0	88.5	..	83.0	82.0	77.5	74.0	70.5	69.5	..	66.0	61.5	59.5	57.0	55.0	46.0	35.5	30.0
A + D	..	..	..	..	89.0	..	80.0	..	..	..	..	..	..	57.5	..	..	36.0	..
I-B = 20 mg. % water A	70.2	..	52.2	46.1	40.0	33.3	30.2	27.1	..	20.5	16.9	12.8	9.2	5.1	..	..	..	..
A + D	..	..	..	50.0	48.9	..	..	..	..	..	..	..	..	9.2	..	..	..	..
I-C = 20 mg. % Water-Alcohol (16%) A	69.4	..	48.7	38.3	23.3	11.9	5.1	..	..	..	..	..	..	..	..	..	..	..
A + D	..	..	..	..	..	16.0	15.5	..	..	..	..	..	..	..	..	..	..	..
I-D = 20 mg. % Simple Syrup	96.3	92.6	90.8	88.5	88.0	..	84.4	82.6	78.9	..	74.3	70.1	..	..	62.4	42.2	..	..
II—Vitamin C + B Vitamins																		
II-B = 50 mg. % water A	..	78.6	74.6	70.5	69.2	67.2	64.7	..	60.6	57.8	55.7	53.5	51.2	50.4	47.1	35.2	16.8	5.0
A + D	..	..	77.9	75.0	..	..	..	..	..	..	..	56.9	..	..	..	..	..	..
II-C = 50 mg. % Water-Alcohol (16%) A	89.7	..	73.4	69.5	61.3	55.3	46.3	42.0	..	26.1	19.3	13.7	..	..	..	..	..	..
A + D	..	..	..	..	..	64.3	..	..	..	..	..	..	..	..	..	..	..	..
II-D = 50 mg. % Simple Syrup	96.9	95.8	93.9	92.7	92.3	..	90.8	89.3	87.4	..	87.0	85.8	..	..	85.4	80.1	75.7	..
III—Vitamin C + Metals																		
III-B = 50 mg. % Water	78.0	52.4	32.4	20.0	15.2	..	7.6	6.4	2.9	1.7	..	..	..	..	..	..	..	..
III-C = 50 mg. % Water-Alcohol (16%)	80.1	..	44.9	24.5	12.0	6.4	4.6	..	..	..	..	..	..	..	..	..	..	..
III-D = 50 mg. % Simple Syrup	84.7	77.7	69.9	73.6	68.9	..	63.2	61.1	58.8	..	53.7	51.1	..	..	47.4	39.8	..	..
IV—Vitamin C + B Vitamins + Metals																		
IV-B = 50 mg. % Water	81.2	65.5	55.0	42.6	33.4	..	25.2	14.9	9.1	5.0	..	..	..	..	..	..	..	..
IV-C = 50 mg. % Water-Alcohol (16%)	81.9	..	54.1	36.1	22.7	14.8	8.8	..	..	..	..	..	..	..	..	..	..	..
IV-D = 50 mg. % Simple Syrup	87.4	85.8	83.5	80.6	76.9	..	73.9	72.0	..	..	70.7	69.8	..	..	64.3	60.6	49.1	..
IV-E = 50 mg. % Simple Syrup Alcohol (16%)	88.8	88.0	85.9	81.8	80.1	..	79.3	73.5	71.0	69.4	68.5	65.8	..	61.2	59.4	47.9	40.3	31.8
V—Influence of *p*H																		
I-A + oxalic acid 200 mg. %	97.5	97.0	..	95.0	95.0	93.5	92.5	91.0	90.0	..	88.5	86.5	86.0	85.0	84.5	80.5	75.5	71.0
I-B + oxalic acid 20 mg. % A	97.5	..	96.0	92.4	89.9	87.4	83.8	80.8	..	76.7	72.2	69.5	67.1	65.1	62.1	45.9	37.2	..
A + D	..	..	..	..	..	..	..	..	..	..	..	..	67.1	..	..	..	37.9	..
A + D	..	..	..	..	..	..	..	..	..	..	..	..	67.1	..	..	..	37.9	..
II-B + oxalic acid 50 mg. %	..	100.0	99.1	97.9	96.2	95.0	92.9	..	90.8	88.3	86.7	85.8	85.0	83.8	..	76.7	70.1	65.5
III-B + citric acid 50 mg. %	92.0	88.0	85.2	78.8	70.8	..	59.6	43.6	34.4	30.8	26.4	18.8	..	8.8	..	..	..	..
IV-B + citric acid 50 mg. %	92.4	88.0	84.8	82.8	76.0	..	68.0	52.8	51.2	38.8	37.2	30.4	..	20.8	14.7	..	..	..
IV-E + citric acid 50 mg. %	94.2	89.9	82.3	78.4	78.4	..	74.0	69.3	67.4	63.3	60.1	58.3	..	45.9	42.1	34.3	23.2	15.8

stoppers and without special precautions. In all cases ordinary distilled water was used.

The figures in the table represent the percentage of ascorbic acid preserved unaltered, with relation to the content of the solution titrated by the same procedure as soon as prepared. That initial concentration is expressed in each case by giving the composition of the solutions in milligrams of vitamin C per 100 cc. of solution (mg. %). Occasionally the sum of the ascorbic acid plus dehydroascorbic acid (A + D), previously reduced with H_2S, was determined. The expression "B Vitamins" represents for each 50 mg. of ascorbic acid: 2 mg. vitamin B_1, 4 mg. vitamin B_2, 6 mg. vitamin B_6, 6 mg. calcium pantothenate, 60 mg. nicotinamide, and 672 mg. lactose. The expression "Metals" represents for each 50 mg. of ascorbic acid: 1.50 Gm. green iron ammonium citrate, 0.05 Gm. cupric citrate, and 0.06 Gm. manganese sulfate. The oxalic acid was used in a concentration of 2%, the citric acid of only 0.8%.

RESULTS

As can be seen in the table, the simple concentration of the vitamin C influences the rate of destruction. The time of semidestruction (T. S. = 50% of the initial quantity unaltered) may be taken as a point of reference. While at a concentration of 0.2% in water alone T. S. = eighteen days, at a concentration ten times less (0.02%) T. S. = three to four days. Possibly this may be an indirect effect of the *p*H at different concentrations. If, in place of pure water as a vehicle, diluted alcohol (16%) is used, the destruction is slightly more rapid (T. S. = two to three days). On the other hand, if for the low concentration of 0.02%, simple syrup is used as vehicle, the destruction is much slower (T. S. = nineteen days).

The destructive effect of the alcohol and the protective effect of the syrup are manifested similarly in the presence of B vitamins (T. S. = fourteen days alone, six to seven days with alcohol, twenty-five days with syrup) and in the presence of metals (T. S. = two to three days alone, two to three days with alcohol, twelve to thirteen days with syrup) for a concentration of 0.05%. From the comparison of both groups of experiments (II–III) is deduced a slight protective effect of the B vitamins which is even more clearly shown by comparing groups III–IV.

In group V, in which all the tests were protected by means of strong acid reaction, the same influence of the concentrations is also noticed (T. S. = thirty days 0.2%, and nineteen days 0.02%). A strong acid reaction can lengthen the time of semidestruction by more than fifteen days if metals which catalyze the oxidation are not present, but even in that case the acid reaction can prolong it five to six days more.

From time to time, the dehydroascorbic acid has been determined. As can be seen, its proportion is minimal, which permits deducing that it is always a question of an integral destruction. The reversible oxidation does not account even partially for the destruction of vitamin C.

It may be concluded that, even in the worst circumstances for accelerating the destruction of vitamin C (aqueous-alcoholic solution, presence of Fe, Cu, and Mn) by combining with protective elements (syrup as vehicle, B vitamins, acid reaction) the time of semidestruction can be deferred until thirteen to fourteen days, conserving 80% unaltered at five days and more than 60% at ten days.

DISCUSSION

The catalytic effect of certain metals on the destruction of vitamin C is well known and has been cited repeatedly since its discovery (1). Differences in the rapidity of oxidation, depending on the concentration, have been mentioned previously (2). Also, there have been mentioned a weak accelerating effect of alcohol (3) and a protective effect of syrup (4), both coincident with our determinations. The protective effect of the acid reaction is exceedingly well known; if oxalic acid may not well be used in pharmaceutical preparations, then citric acid, to which is attributed a greater protective effect than to tartaric acid, may be employed (5).

On the other hand, it has been mentioned that vitamin B_2 accelerates the destruction of vitamin C (6). Our experience with a combination of B vitamins—among them B_2—indicates, on the contrary, a stabilizing effect.

SUMMARY

The stability of vitamin C has been determined in aqueous solutions, expressed in the number of days required for destruction of 50 per cent (time of semidestruction), and combined with various factors accelerating the oxidation (dilution, alcohol, metals, elevation of *p*H) and with other protective factors (concentration, syrup, B vitamins, reduction of *p*H).

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Pharmacognostical Studies of Four American Arnicas*

By HEBER W. YOUNGKEN† and ELMER H. WIRTH‡

Reported are the results of studies of four additional American Arnicas: *Arnica Chamissonis* Less. subsp. *genuina* Maguire, *Arnica Chamissonis foliosa* (Nuttall) Maguire, *Arnica mollis* Hooker var. *genuina* Maguire, and *Arnica longifolia* D. C. Eaton subsp. *genuina* Maguire. The gross anatomical and histological characteristics of the flower heads and their parts and pollen are described and illustrated. Observations are also reported on the plants from which the authentic flower heads were obtained.

IN AN earlier paper published in THIS JOURNAL (1), the authors presented the results of their studies of *Arnica montana* (European Arnica) and of three American species of *Arnica* (*Arnica fulgens*, *A. sororia*, and *A. cordifolia*) which yielded considerable of the Arnica Flowers then on the American market. Flower heads of other American species of *Arnica* have occurred in American commerce, some of which may be found equal to or superior in properties to those described in our previous paper and which have been given official recognition in the National Formulary. With a view toward establishing criteria whereby additional American Arnicas might be determined, we have extended our studies, and, in this paper, report our observations on four additional kinds, namely, *Arnica Chamissonis* subsp. *genuina*, *Arnica Chamissonis* subsp. *foliosa*, *Arnica mollis* var. *genuina* and *Arnica longifolia* subsp. *genuina*.

MATERIALS AND METHODS

The materials used in this study comprised herbarium sheets and flower heads separated therefrom of *Arnica Chamissonis* Lessing subspecies *genuina* Maguire, collected by J. M. Macoun at Buffaso Prairie, Jasper Park, Alberta, July 31, 1918; of *Arnica Chamissonis* Lessing subspecie *foliosa* (Nuttall) Maguire, collected by S. B. Parish in Bear Valley, San Bernardino Mts., San Bernardino Co., Calif., June 16–20, 1895; by E. B. and L. B. Payson at Kendall, Sublette Co., Wyo., Aug. 5, 1922; and by E. C. Moran on rocks of McDonald Pass, Mont., July 16, 1942; of *Arnica mollis* Hooker, collected by E. C. Moran at King's Hill, Belt Mts., July 19, 1942; of *Arnica mollis* Hooker var. *genuina* Maguire, collected by J. W. Thompson, Chelan Co., Wash., Aug. 10, 1935; and of *Arnica longifolia* D. C. Eaton subspecies *genuina* Maguire, collected by Bassett Maguire and A. H. Holmgren in Elko Co., Nev., Aug. 18, 1943; and by Frank W. Gould at Forsyth Canyon, Utah, on shaded stream bank, July 23, 1942, all of which were verified by Bassett Maguire, also about 4 pounds of dried flower heads of "*Arnica foliosa*" and "*Arnica mollis*" both of which were gathered by E. C. Moran in Big Horn Mts., Wyo., during the summer of 1945, and numerous additional sheets of these plants from other collectors and stations in the Gray Herbarium.

The herbarium sheets were checked with the descriptions of the respective Arnicas given by Maguire in his "Monograph of the Genus *Arnica*" and found to agree in respect to their gross essential characters. The gross morphological characters of each of these plants including measurements of their stems, leaves, and flower heads were recorded, and sufficient flower head materials for more detailed microscopic examination were separated from the mounted sheets. Each of the different kinds of flower heads were then separately macerated in water, transferred to labeled Syracuse watch glasses, and dissected on the stage of a binocular dissecting microscope, their parts transferred to labeled vials containing a preserving solution of water, glycerin, and alcohol, from which they were later transferred to microslides, mounted, and studied under the low- and high-power objectives of compound microscopes. A number of the commercial flower heads labeled "*Arnica foliosa*" and "*Arnica mollis*" were macerated, dissected, their parts separately mounted and similarly studied. Measurements were made with the metric rule and standardized ocular micrometer. Photographs were made of the herbarium specimens and drawings prepared of the mounted dissected parts of the flower heads of the subspecies discussed in this paper to show their comparative histological characters.

PLANTS

Arnica Chamissonis is a large group of perennial herbs having probably the greatest extent of distribution and the largest variety of forms of any species of *Arnica* occurring in North America.

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Fig. 1.—*Arnica Chamissonis* Lessing subspecies *genuina* Maguire.

This species was originally described by Lessing (3) in "*Linnaea*" who called attention to the nearness of the species to *Arnica montana*. Maguire studied it in numerous American stations and noted sufficient differences between its forms in different geographical regions of western North America to lead him to segregate the species into three subspecies on the basis of morphological and geographical considerations. The concepts upon which he established these subspecies are stated in his "Monograph of the Genus *Arnica*" (2), as follows:

Arnica Chamissonis Lessing subsp. *genuina* Maguire. Periclinium densely villose with coarse moniliform hairs intermixed with dense, tawny, stipitate glands; pappus mostly sub-plumose, leaves all sessile or the lowermost short petiolate, the margin mostly serrate-dentate.

Arnica Chamissonis Lessing subsp. *foliosa* (Nuttall) Maguire. Pilosity of periclinium predominatingly merely septate, but not at all moniliform; pappus mostly merely barbellate; lower cauline leaves characteristically petiolate, the margin entire, denticulate or occasionally somewhat dentate-serrate.

Arnica Chamissonis Lessing subsp. *incana* Maguire. Plants silvery pilose and tomentose, of wet habitats in the Sierra Nevada and Cascade Ranges.

Arnica Chamissonis Lessing subsp. *genuina* Maguire (Fig. 1) is a perennial herb whose underground portion consists of a subcylindrical, oblique rhizome up to 30 cm. in length and up to about 3 mm. in diameter, bearing short scales at the nodes and numerous slender rootlets. Its overground portion consists of an erect, longitudinally striated, red-tinged stem, puberulent to canescent with clothing and glandular hairs, becoming increasingly glandular-puberulent above and terminating in a corymb of from 3 to 9 flower heads of ligulate and tubular florets having yellow corollas. The stem bears from 5 to 7 pairs of ovate, lanceolate, or oblanceolate cauline leaves, short-hairy on both surfaces and with acute to obtuse apex, dentate-serrate or denticulate margin, sessile above, petiolate with short-winged petioles below. In the herbarium material examined, the leaves attained a length of 8 cm. and a width of about 3 cm. The peduncles were white-hairy. The fruit is an oblong achene, the pappus subplumose.

Maguire (2) gives its geographical distribution and habitat as follows: "In wet meadow lands and moist shaded places in deep soil; in the islands of the Alaskan Archipelago and on the mainland; passing into the subspecies *foliosa* in Northern Washington and Montana and toward the east in the Northwest Territories, eastern Alberta and Manitoba."

Arnica Chamissonis Lessing subsp. *foliosa* (Nuttall) Maguire (2) is a perennial herb first described by Nuttall (4) under the name of *Arnica foliosa*, in 1841. Its underground portion consists of slender, elongated rhizomes similar to the former subspecies from which emanate erect pubescent stems attaining a length of 9 dm. bearing opposite, lanceolate, oblong-lanceolate to oblanceolate, short-hairy leaves with mostly acute apex and entire to irregularly denticulate or rarely serrate-dentate margins, sessile above, but petiolate and sheathing below and forming characteristic ochreae at their bases. From the summit of the stem and axils of upper cauline leaves arise slender, glandular pubescent peduncles, attaining a length of 5 cm. in the materials examined, bearing hemispheric-campanulate flower heads whose corollas are pale yellow. The fruit is an oblong achene. The pappus is barbellate. Its geographical distribution and habitat, as reported by Maguire (2), is in the Northwest Territories and eastern Alberta to Manitoba, westward to Montana and Washington, thence south to San Bernandine Mts. of California, in the southern Rocky Mts. to northern New Mexico, and on the alluvial flats of the Colorado of the West.

The herbarium sheets of this subspecies which we examined showed the radical leaves to be fascicled, petiolate, the blades being lanceolate, irregularly denticulate, with acute apex and up to 15 cm. in length and 2 cm. in width. The cauline leaves were sessile, entire to irregularly denticulate, acute, up to 8 cm. in length and up to 18 mm. in width. The involucral bracts were less villose-hairy than those of the subspecies *genuina* and their tips were tinged with red.

Arnica mollis Hooker (5), including var. *genuina* (Fig. 3), of Hairy Arnica is a perennial herb whose subterranean portion consists of a branching rhizome up to 4 mm. in thickness bearing fibrous rootlets. From each rhizome branch arises a single, erect, longitudinally striate, glandular-puberulent stem bearing from 1 to 3, occasionally up to 6 heads at its summit. Its radical leaves are oblong-lanceolate to oblanceolate and petiolate. Its cauline leaves are arranged in 2 to 4 pairs, sessile above, petiolate below; the lamina ovate, elliptic lanceolate, obovate to oblanceolate, its apex acute or obtuse, its margin irregularly denticulate or serrulate or nearly entire. The flower heads, usually up to 3, possess bright yellow to orange corollas. The involucral bracts are often purplish-tinged toward the apex. The fruit is a glandular-hairy, oblong achene. The pappus is very pale brown and plumose.

Maguire (2) gives its distribution and habitat in the Rocky Mts. from Alberta and British Columbia south to Colorado and Utah and in the Cascade and Sierra Nevada Ranges south to Central California. Robinson and Fernald (6) extend the species eastward, giving its distribution from E. Quebec to British Columbia, south to the mountains of Maine and New Hampshire, Colorado and California.

Arnica longifolia D. C. Eaton (7) is a perennial herb occurring in dense colonies (Fig. 4). Its rhizome is short, up to 5 mm. in diameter, and considerably branched. Its aerial portion consists of dense clumps of minutely scabrous leaf and flowering stems. The stems are longitudinally striate and terminate in cymes of from 3 to 20 turbinate-campanulate flower heads of yellow florets. The leaves

Fig. 2.—*Arnica Chamissonis* Lessing subspecies *foliosa* (Nuttall) Maguire.

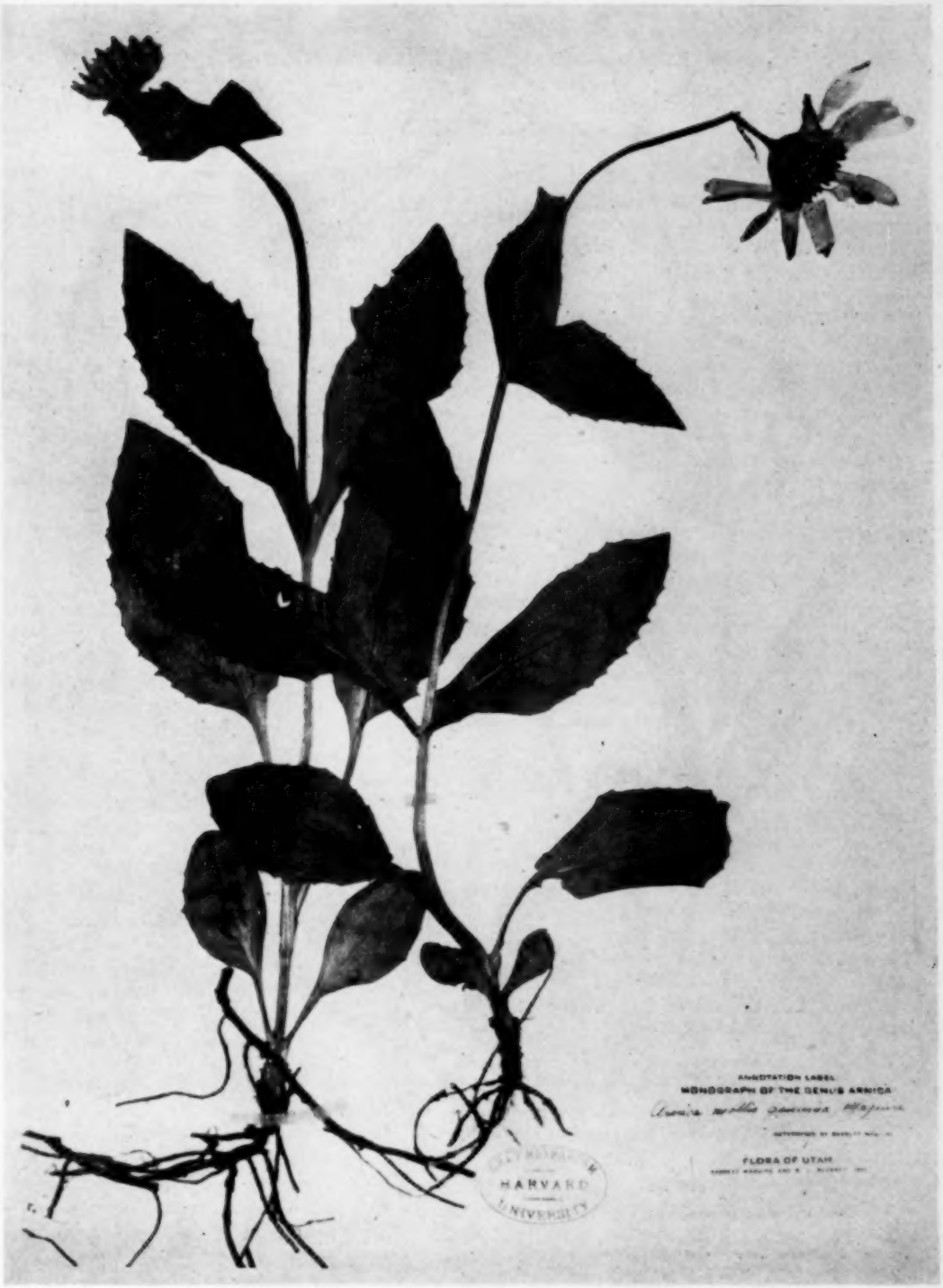

Fig. 3.—*Arnica mollis* Hooker var. *genuina* Maguire.

Fig. 4.—*Arnica longifolia* D. C. Eaton subspecies *genuina* Maguire.

vary from ochreate scales on the lower part of the stem to those of typical foliage type above, the latter in 5 to 7 pairs, and up to about 15 cm. in length. They are lanceolate to elliptic-lanceolate with acute apex, entire or denticulate margin, puberulous and glandular-punctate, sessile to subsessile above and narrowed below to a sheathing connate base. The fruits are oblong achenes narrowed toward the base. The pappus is barbellate to subplumose. Maguire (2) gives its range and habitat as in the higher mountains of Washington, Idaho, and Montana, south in the Cascade and Sierra Nevada Ranges into California, in the Rocky Mts. into Colorado and Utah, on the banks of the upper Gray Run in Salt River Mts., Lincoln Co., Wyo. On the basis of geographical and morphological considerations, he has separated this species into the subspecies *genuina* and *myriadenia*, giving as the key differences the following: *Arnica longifolia* D. C. Eaton subsp. *genuina* Maguire. Pappus stramineous, barbellate; periclinium puberulent and short stipitate-glandular; plants of the Rocky Mts. *Arnica longifolia* D. C. Eaton subsp. *myriadenia* Maguire. Pappus mostly tawny, subplumose; periclinium sparsely moniliform pilose as well as puberulent and short stipitate-glandular; plants mostly of the Cascade and Sierra Nevada Ranges.

Gross Structure and Histology of the Flower Heads

Arnica Chamissonis genuina.—(Fig. 5) Heads hemispheric-campanulate, up to 18 mm. in height; receptacle hemispherical, convex, and deeply pitted; peduncle showing herbaceous dicotyl structure; its epidermis consisting of longitudinally elongated epidermal cells, stomata, and numerous uniseriate, nonglandular hairs of short, dagger type and longer articulate type, the latter being both narrow and broad usually up to 7 cells, occasionally up to 13 cells and up to 1103 μ in length, also glandular hairs with uni- to biseriate stalks and 1- to 2-celled heads, up to 130 μ in length; its cortex narrow; its many, short fibrovascular bundles being capped externally by crescentic arcs of lignified pericyclic fibers, and its pith broad and parenchymatous. Periclinium (involucre) of two series of lanceolate bracts. Outer bracts up to 12 mm. long and 3 mm. broad, their summits acute and pilose-tipped, their nonglandular hairs numerous, consisting of both unicellular and uniseriate types with usually sharp pointed ends, the unicellular type up to 1400 μ in length, the uniseriate type of two kinds: short, dagger-shaped hairs of 1 to 3 cells and long, articulate (moniliform) hairs of up to 10 cells, the basal cell or cells being short, the distal cell elongated. Inner bracts up to 10 mm. long and 1.2 mm. in width with acute to acuminate tips which are pilose with long, mostly unicellular hairs, although an occasional 2-celled, uniseriate hair is found with short basal cell and very long distal cell, the hairs at tip up to 1250 μ in length, the marginal, articular hairs up to 2240 μ in length; glandular hairs up to 60 μ in length with uniseriate to biseriate stalks and unicellular to multicellular heads occur on both kinds of bracts. Ligulate florets up to 21 mm. in length, length of ligula up to 17 mm. of achene up to about 4 mm.; lobes of ligula 3, frequently irregular in length with rounded summits, the maximum length of lobes being 1040 μ; veins in ligula, usually 7, but up to 10; hairs on ligulate corolla nonglandular and glandular, the nonglandular hairs on tube up to 9-celled, those on ligula up to 7-celled, mostly uniseriate, the articulate type with usually long distal cells and up to 980 μ in length; glandular hairs on tube and ligula up to 61 μ in length with an usually short uni- to biseriate stalk and a uni-, bi-, to multicellular-head; achene of ligulate floret oblong, constricted near basal end which is slightly concave, its epidermis with numerous twin hairs up to 210 μ in length and glandular hairs similar to those on the corolla, phytomelane occurring in irregular, branched streaks beneath the epidermis. Pappus of ligulate floret, a circle of subplumose bristles up to about 5 mm. in length with more or less divergent setal hairs mostly from 120 to 304 μ in length, the terminal setal hairs from 1 to 4, compact.

Tubular florets up to 15 mm. in length, their achenes up to 5 mm. long, oblong, tapered toward the base which is discoidal; the epidermis of the tubular corolla bearing numerous nonglandular hairs largely of the uniseriate-articulate type with short basal cells and long distal cell, usually up to 5-celled, occasionally of 6 or 7 cells, and a few short glandular hairs; corolla lobes with papillose epidermis and showing unicellular to uniseriate, rigid nonglandular hairs on their under surface; achenes with twin hairs up to 225 μ in length, an occasional unicellular or uniseriate articular nonglandular hair in the upper part and few scattered glandular hairs with a uniseriate stalk and 1- to 2-celled head, up to 76 μ in length; pappus subplumose, from four-fifths to five-sixths the length of the corolla, up to 6 mm. in length, of a circle of barbellate to subplumose bristles with mostly divergent setal hairs, the terminal setal hairs ranging from 1 to 3, rarely 4, and up to 600 μ in length; pollen grains spherical, spinose, and up to 40 μ in diameter; phytomelane present in subepidermal tissue of achene.

Arnica Chamissonis foliosa.—(Fig. 5) Flower heads hemispheric-campanulate, up to 17 mm. in height and 40 mm. in width. Receptacle hemispherical and deeply pitted, its surface with numerous slender unicellular and 2- to 3-celled, uniseriate, articulate, nonglandular hairs up to 640 μ. Peduncle glandular puberulent with numerous unicellular nonglandular hairs, some of which are short and conical with rounded apex, others narrow and elongate and frequently curved, numerous articulate nonglandular hairs up to 5-celled with short basal cell or cells and elongated, pointed distal cell, and numerous glandular hairs up to 120 μ with uni- to biseriate stalk and a 1- to 2-celled head. Periclinium biseriate; bracts ovate-lanceolate to broadly ovate-lanceolate,

obtuse to nearly acute, pilose at tip, up to 9 mm. in length and up to 3 mm. in width, their surfaces with numerous nonglandular and glandular hairs, the nonglandular straight and curved, the unicellular type narrow and broad in variety, those of the pilose tips frequently up to 650 μ, occasionally up to 900 μ in length, those along the margin up to 390 μ, the uniseriate type up to 5-celled with short basal cell and long distal cell, the marginal ones up to 750 μ in length, a few straight to curved, mostly appressed dagger-shaped hairs; the bract glandular hairs frequently club-shaped, with short, uniseriate stalks and 1- to 2-celled heads, and up to 48 μ in length. Ligulate florets up to 17 mm. in length, the ligula pale yellow, 3-toothed, 7-veined, and up to 13 mm. in length, the lobes elongate dome-shaped; hairs mostly on corolla tube, few on ligula, the nonglandular hairs unicellular and uniseriate-articular, the latter usually up to 6-celled, with short basal cell or cells and very long, frequently curved, distal cell, and up to 975 μ in length, the glandular hairs with short, uniseriate stalks and 1- to 2-celled heads; ligu-

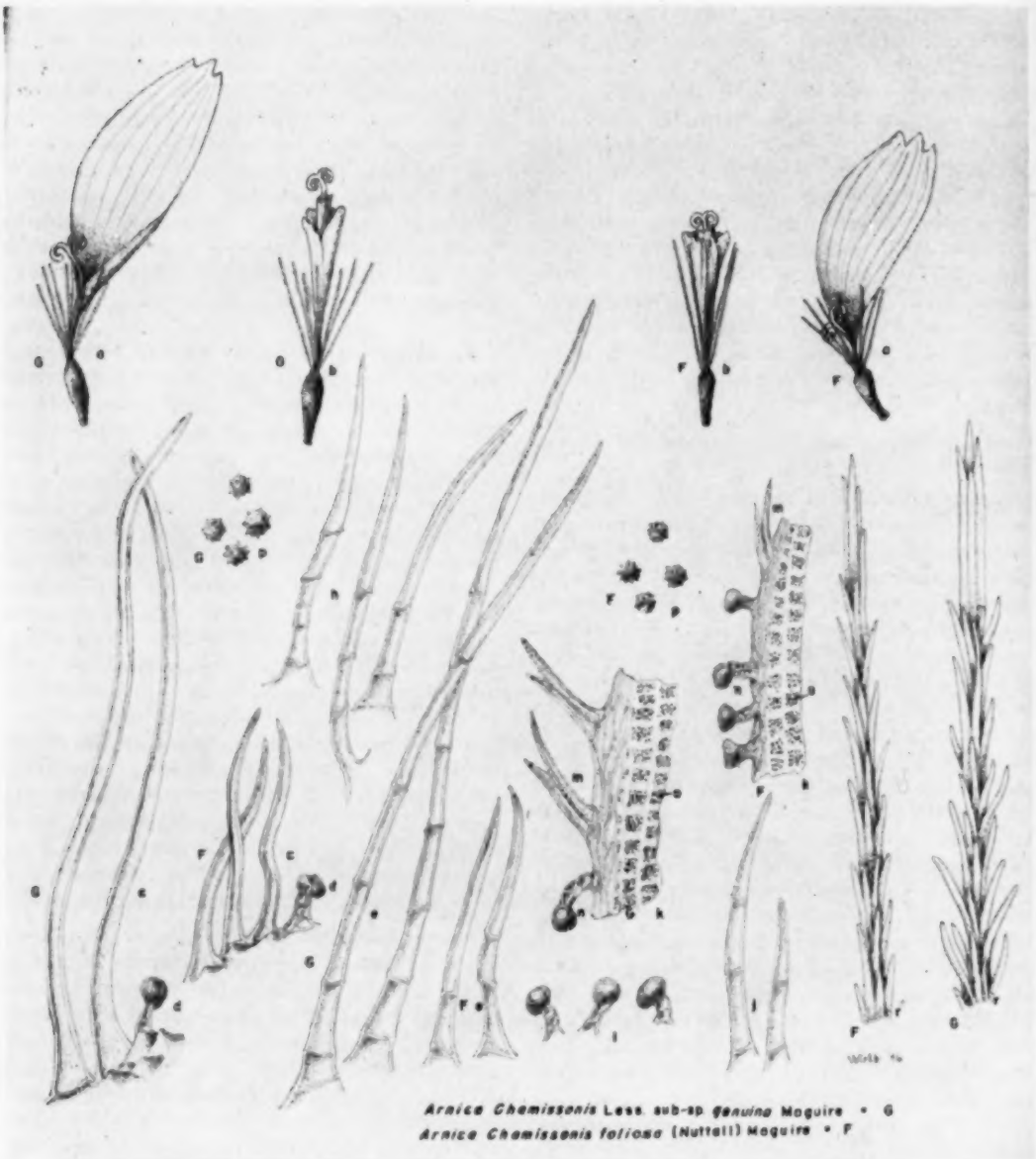

Fig. 5.—*Arnica Chamissonis* Lessing subspecies *genuina* Maguire (G); *Arnica Chamissonis foliosa* (Nuttall) Maguire (F). *a*, Ligulate floret; *b*, tubular floret; *c*, terminal hairs of bracts; *d*, glandular hairs of bracts; *e*, marginal hairs of bracts; *h*, nonglandular hairs of ligulate and tubular floret corollas; *i*, glandular hairs of ligulate and tubular floret corollas; *j*, nonglandular hairs of receptacle; *k*, surface of achene; *m*, twin hairs of achene; *n*, glandular hairs of achene; *o*, phytomelane; *p*, pollen; *r*, terminal hairs of pappus bristle. Ligulate and tubular florets ×2.3. All other elements ×77.

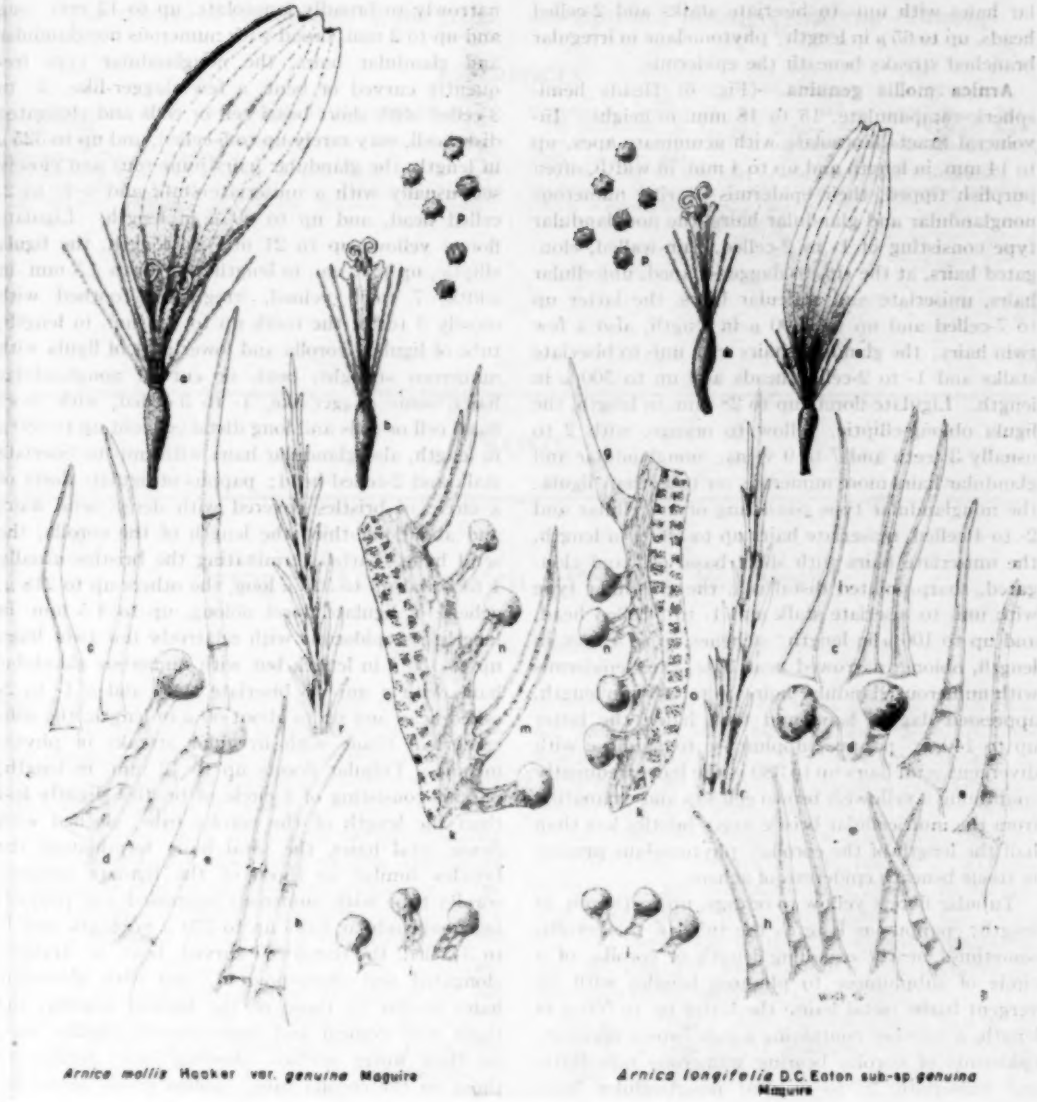

Fig. 6.—*Arnica mollis* Hooker var. *genuina* Maguire and *Arnica longifolia* D. C. Eaton subspecies *genuina* Maguire. *a*, Ligulate floret; *b*, tubular floret; *c*, terminal hairs of bract; *d*, glandular hairs of bract; *e*, marginal hairs of bract; *g*, nonglandular hairs of achene; *h*, nonglandular hairs of ligulate and tubular floret corollas; *i*, glandular hairs of ligulate and tubular floret corollas; *j*, nonglandular hairs of receptacle; *k*, surface of achene of tubular floret; *m*, twin hairs of achene; *n*, glandular hairs of achene; *o*, phytomelane; *p*, pollen; *r*, terminal portion of a pappus bristle. Ligulate and tubular florets ×2.3. All other elements ×77.

late achene oblong, up to 4 mm. in length, tapered toward the base, its surface with or without twin hairs up to 127 μ and with glandular hairs abundant having mostly biseriate stalks and 1- to 2-celled heads, and up to 60 μ in length; phytomelane in irregular streaks beneath the epidermis; pappus of ligulate floret of a circle of barbellate to subplumose bristles almost half the length of the corolla, each bristle with 1 to 3 terminal setal hairs, the latter up to 266 μ in length. Tubular florets from 7.5 to 12.5 mm. in length; the corolla with numerous unicellular and articulate nonglandular hairs, the latter up to 7-celled and up to 850 μ in length, the teeth of the limb bearing papillae and nonglandular hairs of from 1 to 3 cells; pappus almost the length of the corolla and consisting of a circle of barbellate bristles up to 10 mm. in length, the terminal hairs of the bristles usually compact and up to 4; pollen grains spheroidal and spinose, and up to 32 μ in diameter; achenes oblong, sometimes slightly curved toward

the base, up to 6 mm. in length, their surface bearing scattered twin hairs up to 127 μ in length, few short conical unicellular hairs, and numerous glandular hairs with uni- to biseriate stalks and 2-celled heads, up to 65 μ in length; phytomelane in irregular branched streaks beneath the epidermis.

Arnica mollis genuina.—(Fig. 6) Heads hemispheric-campanulate, 15 to 18 mm. in height. Involucral bracts lanceolate with acuminate apex, up to 14 mm. in length and up to 4 mm. in width, often purplish tipped, their epidermis bearing numerous nonglandular and glandular hairs, the nonglandular type consisting of 1- to 2-celled, thin-walled, elongated hairs, at the tip, to dagger-shaped, unicellular hairs, uniseriate and articular hairs, the latter up to 7-celled and up to 1300 μ in length, also a few twin hairs; the glandular hairs with uni- to biseriate stalks and 1- to 2-celled heads and up to 500 μ in length. Ligulate florets up to 28 mm. in length, the ligula oblong-elliptic, yellow to orange, with 2 to usually 3 teeth and 7 to 9 veins; nonglandular and glandular hairs more numerous on tube than ligula; the nonglandular type consisting of unicellular and 2- to 4-celled, uniseriate hairs up to 500 μ in length, the uniseriate hairs with short basal cell and elongated, sharp-pointed distal cell, the glandular type with uni- to biseriate stalk and 1- to 2-celled head, and up to 100 μ in length; achenes up to 4 mm. in length, oblong, narrowed near base, their epidermis with numerous glandular hairs up to 140 μ in length, appressed dagger hairs and twin hairs, the latter up to 140 μ; pappus subplumose to plumose with divergent setal hairs up to 380 μ, the hairs frequently containing a yellowish brown cell sap and emanating from the multicellular bristle axis; bristles less than half the length of the corolla; phytomelane present in tissue beneath epidermis of achene.

Tubular florets yellow to orange, up to 18 mm. in length; pappus as long as the tube of the corolla, sometimes nearly equalling length of corolla, of a circle of subplumose to plumose bristles with divergent barbs (setal hairs) the latter up to 500 μ in length, a number containing a pale brown pigment; epidermis of corolla bearing numerous unicellular and uniseriate, 2- to 4-celled nonglandular hairs up to 500 μ in length and glandular hairs with uni- to biseriate stalks and 1- to 2-celled glandular heads up to 114 μ; lobes of corolla with simple to branched papillae and frequently showing projecting, rigid, dagger hairs on their under surface; achenes up to 6 mm. in length, oblong, narrowed above base and discoid at base, their surface with twin hairs and dagger hairs up to 245 μ in length and glandular hairs up to 140 μ in length; phytomelane present in subepidermal tissue; pollen grains up to 38 μ in diameter.

Arnica longifolia genuina.—(Fig. 6) Flower head turbinate-campanulate, up to 15 mm. in height. Involucre scantily puberulent. Bracts of involucre narrowly to broadly lanceolate, up to 12 mm. long and up to 2 mm. broad with numerous nonglandular and glandular hairs, the nonglandular type frequently curved or bent, a few dagger-like, 2- to 3-celled with short basal cell or cells and elongated distal cell, very rarely up to 5-celled, and up to 325 μ in length, the glandular hairs numerous and closely set, usually with a uniseriate stalk and a 1- to 2-celled head, and up to 80 μ in length. Ligulate florets yellow, up to 21 mm. in length, the ligula elliptic, up to 2 cm. in length and up to 4.5 mm. in width, 7 to 9 veined, irregularly toothed with mostly 3 teeth, the teeth up to 1.5 mm. in length; tube of ligulate corolla and lower part of ligula with numerous straight, bent, or curved nonglandular hairs, some dagger-like, 1- to 3-celled, with short basal cell or cells and long distal cell and up to 380 μ in length, also glandular hairs with uni- to biseriate stalk and 2-celled head; pappus of ligulate floret of a circle of bristles covered with dense setal hairs and about one-third the length of the corolla, the setal hairs (barbs) terminating the bristles usually 1 to 3 and up to 325 μ long, the others up to 218 μ; achene of ligulate floret oblong, up to 4.5 mm. in length, its epidermis with relatively few twin hairs up to 150 μ in length but with numerous glandular hairs with a uni- to biseriate stalk and a 1- to 2-celled head and up to about 60 μ in length, the subepidermal tissue with irregular streaks of phytomelane. Tubular florets up to 12 mm. in length; pappus consisting of a circle of bristles slightly less than the length of the corolla tube, clothed with dense setal hairs, the setal hairs terminating the bristles similar to those of the ligulate pappus; corolla tube with numerous appressed and projecting nonglandular hairs up to 350 μ in length and 1- to 3-celled, the distal cell curved, bent, or straight, elongated and sharp-pointed, also with glandular hairs similar to those of the ligulate corolla, the teeth with conical and dome-shaped papillae and, on their lower surface, showing hairs similar to those on the corolla tube; pollen grains spherical, spinose, and up to 38 μ in diameter; achene of tubular floret oblong, sometimes curved, with a truncate base, up to 5.5 mm. in length, its surface showing few twin hairs up to 385 μ, or twin hairs absent, and numerous glandular hairs with uniseriate to mostly biseriate stalks and 1- to 2-celled heads, and up to 95 μ in length; phytomelane present in the subepidermis. Receptacle of the flower head low dome-shaped, its surface pitted and bearing numerous dagger-shaped and articular hairs, the latter up to 5-celled and up to 487 μ in length.

SUMMARY

1. Descriptions are given of the verified plants yielding flower heads used in this investigation namely: *Arnica Chamissonis* Lessing subsp. *genuina* Maguire, *Arnica Chamissonis* Lessing subsp. *foliosa* (Nuttall) Maguire, *Arnica mollis* Hooker (including

the var. *genuina* Maguire), and *Arnica longifolia* D. C. Eaton subspecies *genuina* Maguire.

2. The results of our studies of the gross structure of the plants and the histology of the flower heads of each of these four American Arnicas are given by detailed descriptions, photographs, and drawings.

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Note

Pyrogen Testing with "Polish" Rabbits*,†

By CARL E. GEORGI‡

WHITE rabbits ordinarily employed in testing of parenteral solutions for pyrogens possess certain distinct disadvantages. The principal objection to their use is the excessive weight which they attain thus requiring (*a*) the use of large quantities of test solution which often must be administered in a cumbersome manner with a large syringe or requiring the refilling of a smaller syringe, (*b*) large quantities of feed which they consume, and (*c*) large quarters to house them. It is not uncommon to find rabbits weighing from 3 to 5 Kg. being employed in pyrogen testing; this necessitates the use of 30.0 to 50.0 ml. (1) of test solution which cannot be conveniently or rapidly administered. A sizeable colony of heavy animals will also consume an appreciable amount of feed but this, however, can be controlled by limiting the intake to 60 to 65 Gm. daily thus controlling the weight of the animal as well (2). This, of course, requires additional attention by an attendant.

For the past fifteen months, we have successfully employed a breed of rabbit known as the "Polish"[1] rabbit. It is a fancy show animal bred from miniature white New Zealand rabbits. The specifications for show purposes are as follows: maximum weight 1586 Gm. and length of ears 6.3 cm. Frequently breeders have on hand animals which cannot qualify for show purposes and those are ones which we have used. The maximum weight at maturity of these culls may approach 1700 Gm. and the ear length may reach 9.0 cm. The ones which we have employed ranged in weight from 1188 Gm. to 1700 Gm. (permitting the use of a 20.0-ml. syringe) and the ear length from 7.0 cm. to 9.0 cm. The marginal ear vein is prominent; a #25 gauge one-inch needle can be used for injection purposes. The ear length is no deterrent. One becomes accustomed to handling the shorter ears.

The space which these rabbits occupy can be reduced to one-half of that ordinarily employed for the maintenance of the usual type of colonies.

The daily feed consumption, when made available in unlimited quantities, averages 50.0 Gm. (Purina Chow pellets) per Kg. of body weight. This is a distinct advantage in small colonies for they can remain unattended over week-ends and holidays.

The writer wishes to call attention to the fact that a lotion of benzyl benzoate (3) has been successfully used in this Laboratory in the control of the rabbit ear mite (*Psoroptes cuniculi* Delafond). It can be applied with a cotton swab and the lotion rubbed into the affected area.

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† This study was supported by The Smith-Dorsey Company, Lincoln, Nebr.
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[1] Obtained from the Green Hutch, through the courtesy of Mrs. James Hrdlicka, Lincoln, Nebr.

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Book Review

Penicillin: Its Properties, Uses, and Preparations. Published by direction of the COUNCIL OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN. The Pharmaceutical Press, 17 Bloomsbury Square, London, W. C. 1, 1946. viii + 199 pp. 13.5 x 21.5 cm. Price 10s. 6d.

Unquestionably the most significant advance in medication was made when penicillin was discovered and made available to the public. As was often the case with war-time discoveries, however, much information regarding the nature and the use of penicillin was necessarily withheld. Although monographs on the therapeutic application have been available, they have emphasized the hospital approach and have been restricted to therapeutics. The pharmacist has needed a well-rounded view of the entire penicillin picture: the bacteriology, chemistry, pharmacology, and especially the pharmacy of this important antibiotic. Nothing as complete as this has been published in America, although a very complete monograph is being written. The book here reviewed was written to meet these needs for the British pharmacists and many American pharmacists will be grateful for a copy on their shelves.

The monograph opens with a historical summary which is followed by a sizeable chapter on commercial manufacture. The basic facts concerning penicillin are given in an understandable fashion. Such factors as submerged cultures vs. surface cultures, freeze drying, modification of the medium, crystalline penicillin, mold strain studies, and patent specifications are briefly discussed. A flow sheet of production of a British firm is included. It is this area of production that will appeal to many Americans and which some will feel is too short. The intensity of effort in increasing the yield of penicillin by changes in the medium, by examining thousands of mold strains, by refining extraction methods, by the use of precursors and other factors is exciting reading.

Chapter III, containing 8 pages, develops briefly the chemistry of penicillin. The synthesis of penicillin was accomplished by Dr. Du Vigneaud too late in 1946 for inclusion in this monograph but the knowledge gained by degradation is sufficient to make this omission of minor consequence.

The following chapter on stability will be very helpful to practical-minded pharmacists. The factors such as hygroscopicity, *p*H, impurities, temperature, bacterial contamination (penicillinase), alcohols, glycerin, and rubber which contribute to the destruction of penicillin are discussed at some length and in a sound fashion.

Chapter V deals with the standard, unit, and methods of assay of penicillin. Not only are British and American methods of bacteriological assay described, but a colorimetric method is also listed. Although chemical methods reported to date have met with somewhat indifferent success, this monograph would have been enhanced by their inclusion. The assays of pharmaceutical preparations are of interest to many pharmacists. Although precise assay directions are not given, the references to the original literature will aid the worker desiring to go further.

The chapters on pharmacology and clinical use of penicillin outline the salient features for the pharmacist. The student of these sections is in a position to talk intelligently with the physician about the uses and limitations of this drug. Naturally there is not the wealth of detail in these sections when compared with monographs limited to this area almost exclusively.

Chapter VIII will have the strongest appeal to the practicing pharmacist. This chapter, composed of nearly 50 pages, is a thorough discussion of the pharmacy and pharmaceutical preparations of penicillin. This section is a complete treatise on the dispensing of the drug. Here will be found 64 formulas for injections, oral preparations, capsules, mixtures, dusting powders, eye drops, nose drops, lozenges, pastilles, ointments, creams, lotions, sprays, snuffs, suppositories, and chewing wafers gleaned from the medical and pharmaceutical literature.

The bibliography, while far from complete, is highly selective and covers 331 papers selected from both British and American journals. British pharmacists will find the list of proprietaries mentioned in the text along with the addresses of British suppliers very helpful.

Due to the close cooperation between British and American antibiotic workers during the war, there is very little conflict in nomenclature. American readers will have to adjust themselves to reading about penicillins I, II, III, and IV rather than F, G, X, and K. This is of little consequence as workers in both countries will probably soon shelve such terminology in favor of more nearly chemical names such as benzyl penicillin.—MELVIN W. GREEN.